Attorney Docket No. 029318-1001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicants:	Douglas HOVEY et al.
Title:	NOVEL FLUTICASONE FORMULATIONS
Appl. No.:	10/768,194
Filing Date:	2/2/2004
Examiner:	Andriae M. HOLT
Art Unit:	1616
Confirmation Number:	3657

BRIEF ON APPEAL

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Sir:

Under the provisions of 37 C.F.R. § 41.37, this Appeal Brief is being filed together with a credit card payment form in the amount of $540.00 covering the 37 C.F.R. 41.20(b)(2) appeal fee. If this fee is deemed to be insufficient, authorization is hereby given to charge any deficiency (or credit any balance) to the undersigned deposit account 19-0741.

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FEDERAL STATUTES

REAL PARTY IN INTEREST

The real party in interest in this appeal is Elan Pharma International Ltd, which is the assignee of the present application as recorded at Reel/Frame numbers 015491/0685.

RELATED APPEALS AND INTERFERENCES

No related appeals or interferences are pending.

STATUS OF CLAIMS

Claims 1-16, 18, 25-26, 45-46, 62-63, 68 and 82-99 are cancelled. Pending claims 17, 19-24, 27-44, 47-61, 64-67 and 69-81 are finally rejected, and are the subject of this appeal. The pending claims are presented in Appendix A of this Brief.

STATUS OF AMENDMENTS

No claim amendments were made in response to the Office Action issued on April 1, 2009. No other amendments or submissions are pending in the application.

SUMMARY OF CLAIMED SUBJECT MATTER

Independent claims 17, 22, 23, 39 and 60 are to be argued in the brief. The relevant citation to the specification is shown in the parentheses below.

Independent claim 17 reads as follows:

17. A sterile filterable **{p. 10, ll. 20-21}** dispersion **{p. 11, ll. 25-26}** comprising:

(a) an aqueous dispersion medium **{p. 35, ll. 14-21}**;

(b) fluticasone particles sufficiently small to pass through a 0.2μm filter **{p. 20, ll. 7-9}**, and have a phase selected from the group consisting of a crystalline phase, an amorphous phase, and a semi-crystalline phase **{p. 29, ll. 11-12}**; and

(c) at least one surface stabilizer adsorbed on the surface of the fluticasone particles **{p. 28, ll. 12-13}**,

wherein the dispersion is sterilized by filtration through a 0.2 μm filter **{p. 20, ll. 7-9}**.

Independent claim 22 reads as follows:

22. A sterile filterable **{p. 10, ll. 20-21}** fluticasone composition comprising:

(a) particles of fluticasone or a salt thereof **{p. 29, ll. 5-6}**, wherein at least 99.9% of the fluticasone particles have a particle size of less than 200 nm **{p. 20, ll. 3-4}**; and

(b) tyloxapol as a surface stabilizer **{p. 29, ll. 24-25}**,

wherein the composition is sterilized by filtration through a 0.2 μm filter **{p. 20, ll. 7-9}**.

Independent claim 23 reads as follows:

23. A nanoparticulate fluticasone composition **{p. 11, ll. 15-16}** comprising:

(a) particles of fluticasone or a salt thereof **{p. 29, ll. 5-6}**, wherein the fluticasone particles have an effective average particle size of less than 150 nm **{p. 8, ll. 16-18; p. 19, ll. 23-29; p. 32, l. 25}**; and

(b) at least one surface stabilizer adsorbed on the surface of the fluticasone particles **{p. 28, ll. 12-13}**,

wherein the composition is sterilized by filtration **{p. 10, ll. 20-21}** through a 0.2μm filter **{p. 20, ll. 7-9}**.

Independent claim 39 reads as follows:

39. A method of making a fluticasone composition **{p. 10, ll. 25-26}** comprising:

contacting fluticasone or a salt thereof with at least one surface stabilizer for a time and under conditions sufficient to provide a particulate fluticasone composition comprising particles of fluticasone **{p. 10, ll. 26-28}** having an effective average particle size of less than 150 nm **{p. 8, ll. 16-18; p. 19, ll. 23-29; p. 32, l. 25}**; and

passing the particulate fluticasone composition through a 0.2μm filter **{p. 20, ll. 7-9}** to sterilize the particulate fluticasone composition **{p. 10, ll. 20-21}**.

Independent claim 39 reads as follows:

60. A method of treating a subject in need of either symptomatic or prophylactic treatment **{p. 39, ll. 28-29}** with a sterile particulate fluticasone composition **{p. 10, ll. 20-21}** comprising the step of administering to the subject an effective amount of the sterile particulate fluticasone composition **{p. 10, ll. 20-21}** sterilized by passing the composition through a 0.2μm filter **{p. 20, ll. 7-9}**, wherein the sterile particulate fluticasone composition **{p. 10, ll. 20-21}** comprises particles of fluticasone or a salt thereof **{p. 29, ll. 5-6}** and at least one surface stabilizer **{p. 28, ll. 12-13}**, wherein the fluticasone particles have an effective average particle size of less than 150 nm **{p. 8, ll. 16-18; p. 19, ll. 23-29; p. 32, l. 25}**.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The rejections to be reviewed on appeal are the following:

1. Rejection of claims 17, 19-24, 28-44, 47, 49-61, 64-67, 69 and 71-81 under 35 U.S.C. §103(a) for allegedly being obvious over U.S. Patent No. 5,747,001 to Wiedmann et al. ("Wiedmann") in view of U.S. Patent No. 6,241,969 to Saidi et al. ("Saidi").

2. Rejection of claims 17, 19-24, 28-44, 47, 49-61, 64-67, 69 and 71-81 under 35 U.S.C. §103(a) for allegedly being obvious over PCT Publication No. WO 96/25918 by Wood et al. ("Wood") in view of Saidi, and in further view of U.S. Patent Application Publication No. US 2003/0073676 by Biggadike et al. ("Biggadike").

ARGUMENT

I. Summary of Appellants' Claimed Invention

Appellants' claimed invention is directed to a nanoparticulate fluticasone dispersion comprising fluticasone particles and at least one surface stabilizer adsorbed on the surface of the fluticasone particles. The fluticasone particles in Appellants' claimed composition is sufficiently small to pass through a 0.2 μm filter for the purposes of sterilization.

As described in Appellants' specification, application of sterile filtration is challenging for conventional, non-nanoparticulate formulations. This is because "micron-sized drug particles . . . are too large to pass through the membrane pores [having a cut-off size of 0.2 μm]" (specification, page 20, lines 13-15). Moreover, sterile filtration cannot be universally applied to all nanoparticulate active agent formulations. This is "because nanoparticulate active agent compositions have a size range, many of the particles of a typical nanoparticulate active agent composition having an average particle size of 200 nm may have a size greater than 200 nm. Such larger particles tend to log the sterile filter. Thus, only nanoparticulate active agent compositions having very small average particle sizes can be sterile filtered" (*id.*, lines 16-20).

Appellants' independent claim 22 prescribes a sterile filterable fluticasone composition in which at least 99.9% of the fluticasone particles have a particle size of less than 200 nm. Appellants' independent claims 23, 39 and 60 are directed to fluticasone compositions in which the fluticasone particles have an effective average particle size of less than 150 nm. As defined by the specification, "an effective average particle size of less than 150 nm" means that at least 50% of the fluticasone particles have a particle size of less than 150 nm. *See* specification, at page 33, paragraph [0112].

Appellants disagree with the Examiner because one skilled in the art would not be able to combine the teachings of the cited reference, as the Examiner asserts, to obtain the claimed

invention. For the reasons detailed below, Appellants respectfully request the Board resolve the rejection under Section 103(a) in favor of the Appellants and reverse the rejection in whole.

II. Rejection over Wiedmann and Saidi

The Examiner expressly acknowledges that "Wiedmann et al. do not teach fluticasone particles or sterile filtration" but asserts that Saidi remedies the deficiencies of Wiedmann. Final Office Action dated November 5, 2009, page 6, first full paragraph.

A. There is no teaching or suggestion that Wiedmann's composition is suitable for sterile filtration.

Specifically, the Examiner contends that "[i]t would have been obvious to one of ordinary skill in the art at the time of invention to combine the teachings of Wiedmann et al. and Saidi et al. and use fluticasone in the formulation" (final Office Action, page 7, lines 14-16). It appears that the Examiner suggests that one skilled in the art would substitute the active agent in Saidi's composition, fluticasone, for the beclomethasone of Wiedmann's composition.

Assuming, *arguendo*, that one skilled in the art would have a reason to substitute the active agent and that an aerosol composition comprising fluticasone nanoparticles can be successfully achieved, there is no teaching or suggestion that such fluticasone aerosol composition can be sterile filtered by passing through a 0.2 μm filter, as evidenced by Wiedmann.

Wiedmann relates to an aerosol comprising droplets of an aqueous dispersion of beclomethasone nanoparticles and a surface modifier on the surface of the beclomethasone nanoparticles. *See* abstract. Wiedmann further discloses that "[t]he droplets in the aerosols typically have a size [of] less than about 50 microns in diameter . . ." (column 2, lines 63-64) and that the beclomethasone particles have an effective average particle size of less than 400 nm (column 10, lines 24-39). There is no teaching or suggestion that Wiedmann's aerosol composition can be sterilized by filtering through a 0.2 μm filter. As disclosed in the

specification, sterile filtration can be difficult for aerosol formulations of nanoparticulate drugs due to the required small particle size of the composition. *See* specification, page 20, paragraph [0061].

B. <u>It is impossible to apply Saidi's sterile filtration technique to Wiedmann's composition to arrive at the claimed invention.</u>

To further bridge the gap between Appellants' claimed invention and the cited references, the Examiner further claims that "[i]t would also have been obvious to one of ordinary skill in the art . . . to use the sterile filtration technique as taught by Saidi et al. in the formulations and process of Wiedmann et al." (final Office Action, page 8, first full paragraph).

As repeatedly submitted in the prior responses, Saidi applies the sterile filtration technique to drug solutions rather than drug dispersions. *See, e.g.*, response filed on June 26, 2009. pages 20-21; and response filed on January 15, 2009, page 20.

Saidi's method requires that the active agent, such as fluticasone, be ***dissolved*** in a surfactant, for example, a vitamin E derivative TPGS. *See* column 5, lines 34-35, 49-52. Moreover, each of working examples 1-4 discloses that the active agent is ***dissolved*** before passing through a 0.22 micron filter. *See* column 9, line 65 through column 11, line 12. In sharp contrast, the claimed invention is directed to dispersions in which fluticasone exists in a particulate form (crystalline, amorphous, or semi-crystalline) rather in a dissolved state.

One skilled in the art would have understood that solutions can easily pass a 0.22 micron filter while a dispersion may cause the filter to clog if the particles in the dispersion is not sufficiently small. Accordingly, one skilled in the art would not have simply applied Saidi's sterile filtration technique, which applies to a solution, to Wiedmann's composition, as the Examiner suggests, in the absence of any indication that Wiedmann's composition can be successfully sterile filtered.

C. The Examiner improperly assigned a nexus between Wiedman's filtration step and Saidi's sterile filtration.

The Examiner asserts that "[o]ne skilled in the art . . . would have been motivated to implement sterile filtration of Saidi et al. instead of simple filtration of Wiedmann et al. because sterilization of formulations is beneficial to recipients" (final Office Action, page 8, first full paragraph). This statement is technically erroneous.

Wiedmann discloses:

> *After attrition is completed, the grinding media is separated from the milled particulate product (in either a dry or liquid dispersion form) using conventional separation techniques, such as by filtration, sieving through a mesh screen, and the like.*

Column 7, lines 18-22. When placed in context, filtration clearly is used by Wiedmann to separate larger particles from smaller particles or solid from liquid, and therefore, is a separation technique. In contrast, the sterile filtration of Saidi used to remove microorganisms is a sterilization technique. No skilled artisan would have confused the concepts of separation technique and sterilization technique. The Examiner can only make the rejection by taking the terms out of their context and arbitrarily assigning an artificial nexus between these concepts merely based on the appearance of the keyword "filtration."

III. Rejection over Wood, Saidi and Biggadike

Although the Examiner cites a different primary reference, Wood, the rejection rationale is essentially the same as that of the rejection based on Wiedmann and Saidi. Thus, the arguments in the foregoing section are incorporated by reference.

A. The combined teachings of Wood and Saidi would not have led one skilled in the art to Appellants' claimed invention.

Similar to Wiedmann, Wood is directed to aerosols comprising nanoparticulate active agent dispersions, which have a droplet size of less than about 50 microns. The particles of the

active agents have an effective average particle size of less than about 400 nm. *See* Wood, abstract; page 3, lines 18-20; and page 10, lines 1-3. In Example 1, Wood demonstrates that the active agent has "a particle size distribution of 0.26 ± 0.13 mm." There is neither evidence that Wood's aerosol compositions are suitable for sterile filtration nor an indication that Saidi's sterile filtration technique can be applied to Wood's compositions.

As discussed *supra*, the Examiner improperly relied on Wood's alleged teaching of filtration because it is a separation technique applied to separate the grinding media from the composition. *See* Wood, page 12, first full paragraph. Wood has no suggestion whatsoever to sterile filter the composition.

B. The teaching of Biggadike, in combination with those of Wood and Saidi, fails to render the claimed invention obvious.

Biggadike is cited for the alleged teaching of tyloxapol as a surface stabilizer for fluticasone. First, Biggadike fails to address the deficiencies of Wood and Saidi as discussed above. Second, as the Examiner correctly points out, Biggadike uses "tyloxapol [as] a preferred surfactant . . . to ***solubilize*** fluticasone and fluticasone esters" (final Office Action, page 15, lines 10-12; emphasis added). The Examiner has failed to established why one skilled in the art would have any reason to select tyloxapol as a surface stabilizer in the claimed invention, which adsorbs on the surface of fluticasone particles to prevent agglomeration or aggregation in view of Biggadike's teaching of using tyloxapol as a solibilizer.

Accordingly, Appellants respectfully request that the Board reverse the rejection under 35 U.S.C. §103(a) in whole.

CONCLUSION

For the reasons discussed above, Appellants respectfully submit that all pending claims are in condition for allowance, and respectfully requests that the rejections be reversed in whole, and that the claims be allowed to issue.

Respectfully submitted,

Date: April 5, 2010

By _____

FOLEY & LARDNER LLP
Customer Number: 31049
Telephone: (202) 672-5538
Facsimile: (202) 672-5399

Michele M. Simkin
Attorney for Applicant
Registration No. 34,717

APPENDIX A: CLAIMS INVOLVED IN APPEAL

1. – 16. (Cancelled)

17. (Previously Presented) A sterile filterable dispersion comprising:

(a) an aqueous dispersion medium;

(b) fluticasone particles sufficiently small to pass through a 0.2μm filter, and have a phase selected from the group consisting of a crystalline phase, an amorphous phase, and a semi-crystalline phase; and

(c) at least one surface stabilizer adsorbed on the surface of the fluticasone particles, wherein the dispersion is sterilized by filtration through a 0.2 μm filter.

18. (Cancelled)

19. (Previously Presented) The sterile filterable fluticasone dispersion of claim 17, wherein the surface stabilizer is tyloxapol.

20. (Previously Presented) The sterile filterable fluticasone dispersion of claim 17, wherein at least 99.9% of the fluticasone particles have a particle size of less than 200 nm.

21. (Previously Presented) The sterile filterable fluticasone dispersion of claim 17, wherein at least 90% of the fluticasone particles have a particle size of less than 130 nm.

22. (Previously Presented) A sterile filterable fluticasone composition comprising:

(a) particles of fluticasone or a salt thereof, wherein at least 99.9% of the fluticasone particles have a particle size of less than 200 nm; and

(b) tyloxapol as a surface stabilizer,

wherein the composition is sterilized by filtration through a 0.2 μm filter.

23. (Previously Presented) A nanoparticulate fluticasone composition comprising:

(a) particles of fluticasone or a salt thereof, wherein the fluticasone particles have an effective average particle size of less than 150 nm; and

(b) at least one surface stabilizer adsorbed on the surface of the fluticasone particles, wherein the composition is sterilized by filtration through a 0.2μm filter.

24. (Previously Presented) The composition of claim 23, wherein the effective average particle size of the fluticasone particles is selected from the group consisting of less than 140 nm, less than 130 nm, less than 120 nm, less than 110 nm, less than 100 nm, less than 90 nm, less than 80 nm, less than 70 nm, less than 60 nm, and less than 50 nm.

25.-26. (Cancelled)

27. (Original) The composition of claim 23 formulated for administration selected from the group consisting of oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

28. (Original) The composition of claim 23 further comprising one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

29. (Previously Presented) The composition of claim 28, wherein the fluticasone particles are present in the composition in an amount selected from the group consisting of from 99.5% to 0.001%, from 95% to 0.1%, and from 90% to 0.5%, by weight, based on the total combined dry weight of the fluticasone and at least one surface stabilizer, not including other excipients.

30. (Previously Presented) The composition of claim 28, wherein the at least one surface stabilizer is present in an amount selected from the group consisting of from 0.5% to 99.999%, from 5.0% to 99.9%, and from 10% to 99.5%, by weight, based on the total combined dry weight of the fluticasone and at least one surface stabilizer, not including other excipients.

31. (Original) The composition of claim 23, comprising at least two surface stabilizers.

32. (Original) The composition of claim 23, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, and an ionic surface stabilizer.

33. (Previously Presented) The composition of claim 32, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oils, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-derivatized phospholipid, PEG-derivatized cholesterol, PEG-derivatized cholesterol, PEG-derivatized vitamin A, PEG-derivatized vitamin E, and random copolymers of vinyl acetate and vinyl pyrrolidone.

34. (Previously Presented) The composition of claim 32, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, a phospholipid, zwitterionic stabilizers, poly-n-methylpyridinium, anthryul pyridinium chloride, chitosan, polylysine, polyvinylimidazole, polybrene, polymethylmethacrylate trimethylammoniumbromide bromide (PMMTMABr), hexyldesyltrimethylammonium bromide (HDMAB), polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, 1,2 Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-[Amino(Polyethylene Glycol)2000] (sodium salt), Poly(2-methacryloxyethyl trimethylammonium bromide), poloxamines, lysozyme, alginic acid, carrageenan, and nonionic, high molecular weight, water-soluble poly(ethylene oxide) polymers.

35. (Previously Presented) The composition of claim 32, wherein the at least one cationic surface stabilizer is selected from the group consisting of cationic lipids, sulfonium, phosphonium, quarternary ammonium compounds, stearyltrimethylammonium chloride, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl $(ethenoxy)_4$ ammonium chloride, lauryl dimethyl $(ethenoxy)_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts,

lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12}, C_{15}, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, polyquaternium 10, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, quaternized ammonium salt polymers, imidazoline, alkyl pyridinium salts, amines, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

36. (Previously Presented) The composition of claim 35, wherein the amine is selected from the group consisting of alkylamines, dialkylamines, alkanolamines, polyethylenepolyamines, N,N-dialkylaminoalkyl acrylates, vinyl pyridine, amine salts, lauryl amine acetate, stearyl amine acetate, alkylpyridinium salt, alkylimidazolium salt, amine oxides, and imide azolinium salts.

37. (Original) The composition of claim 34, wherein the cationic surface stabilizer is a nonpolymeric compound selected from the group consisting of benzalkonium chloride, a carbonium compound, a phosphonium compound, an oxonium compound, a halonium compound, a cationic organometallic compound, a quarternary phosphorous compound, a pyridinium compound, an anilinium compound, an ammonium compound, a hydroxylammonium

compound, a primary ammonium compound, a secondary ammonium compound, a tertiary ammonium compound, behenalkonium chloride, benzethonium chloride, cetylpyridinium chloride, behentrimonium chloride, lauralkonium chloride, cetalkonium chloride, cetrimonium bromide, cetrimonium chloride, cethylamine hydrofluoride, chlorallylmethenamine chloride (Quaternium-15), distearyldimonium chloride (Quaternium-5), dodecyl dimethyl ethylbenzyl ammonium chloride(Quaternium-14), Quaternium-22, Quaternium-26, Quaternium-18 hectorite, dimethylaminoethylchloride hydrochloride, cysteine hydrochloride, diethanolammonium POE (10) oletyl ether phosphate, diethanolammonium POE (3)oleyl ether phosphate, tallow alkonium chloride, dimethyl dioctadecylammoniumbentonite, stearalkonium chloride, domiphen bromide, denatonium benzoate, myristalkonium chloride, laurtrimonium chloride, ethylenediamine dihydrochloride, guanidine hydrochloride, pyridoxine HCl, iofetamine hydrochloride, meglumine hydrochloride, methylbenzethonium chloride, myrtrimonium bromide, oleyltrimonium chloride, polyquaternium-1, procainehydrochloride, cocobetaine, stearalkonium bentonite, stearalkoniumhectonite, stearyl trihydroxyethyl propylenediamine dihydrofluoride, tallowtrimonium chloride, and hexadecyltrimethyl ammonium bromide.

38. (Original) The composition according to any of claims 32, 34, 35, 36, or 37, wherein the composition is bioadhesive.

39. (Previously Presented) A method of making a fluticasone composition comprising:

contacting fluticasone or a salt thereof with at least one surface stabilizer for a time and under conditions sufficient to provide a particulate fluticasone composition comprising particles of fluticasone having an effective average particle size of less than 150 nm; and

passing the particulate fluticasone composition through a 0.2μm filter to sterilize the particulate fluticasone composition.

40. (Original) The method of claim 39, wherein said contacting comprises grinding.

41. (Original) The method of claim 40, wherein said grinding comprises wet grinding.

42. (Original) The method of claim 39, wherein said contacting comprises homogenizing.

43. (Original) The method of claim 39, wherein said contacting comprises:

(a) dissolving the fluticasone particles in a solvent;

(b) adding the resulting fluticasone solution to a solution comprising at least one surface stabilizer; and

(c) precipitating the solubilized fluticasone having at least one surface stabilizer by the addition thereto of a non-solvent.

44. (Previously Presented) The method of claim 39, wherein the effective average particle size of the fluticasone particles is selected from the group consisting of less than 140 nm, less than 130 nm, less than 120 nm, less than 110 nm, less than 100 nm, less than 90 nm, less than 80 nm, less than 70 nm, less than 60 nm, and less than 50 nm.

45.-46. (Cancelled)

47. (Previously Presented) The method of claim 39, wherein the fluticasone has a phase selected from the group consisting of a crystalline phase, an amorphous phase, and a semi-crystalline phase.

48. (Previously Presented) The method of claim 39 further comprising formulating the particulate fluticasone composition into a dosage form suitable for administration to a patient, wherein the route of administration is selected from the group consisting of oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

49. (Previously Presented) The method of claim 39, wherein the contacting step further comprises contacting the fluticasone with one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

50. (Previously Presented) The method of claim 49, wherein the fluticasone particles are present in the particulate fluticasone composition an amount selected from the group consisting of from 99.5% to 0.001%, from 95% to 0.1%, and from 90% to 0.5%, by weight, based on the total combined dry weight of the fluticasone particles and the at least one surface stabilizer, not including other excipients.

51. (Previously Presented) The method of claim 49, wherein the at least one surface stabilizer is present in the particulate fluticasone composition in an amount selected from the group consisting of from 0.5% to 99.999%, from 5.0% to 99.9%, and from 10% to 99.5%, by weight, based on the total combined dry weight of the fluticasone and the at least one surface stabilizer, not including other excipients.

52. (Original) The method of claim 39, wherein the fluticasone composition comprises at least two surface stabilizers.

53. (Original) The method of claim 39, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, and an ionic surface stabilizer.

54. (Previously Presented) The method of claim 53, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oils, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate,

carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-derivatized phospholipid, PEG-derivatized cholesterol, PEG-derivatized cholesterol, PEG-derivatized vitamin A, PEG-derivatized vitamin E, and random copolymers of vinyl acetate and vinyl pyrrolidone.

55. (Previously Presented) The method of claim 53, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, a phospholipid, zwitterionic stabilizers, poly-n-methylpyridinium, anthryul pyridinium chloride, chitosan, polylysine, polyvinylimidazole, polybrene, polymethylmethacrylate trimethylammoniumbromide bromide (PMMTMABr), hexyldesyltrimethylammonium bromide (HDMAB), polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, 1,2 Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-[Amino(Polyethylene Glycol)2000] (sodium salt), Poly(2-methacryloxyethyl trimethylammonium bromide), poloxamines, lysozyme, alginic acid, carrageenan, and nonionic, high molecular weight, water-soluble poly(ethylene oxide) polymers.

56. (Previously Presented) The method of claim 53, wherein the at least one cationic surface stabilizer is selected from the group consisting of cationic lipids, sulfonium,

phosphonium, quarternary ammonium compounds, stearyltrimethylammonium chloride, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl $(ethenoxy)_4$ ammonium chloride, lauryl dimethyl $(ethenoxy)_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12}, C_{15}, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, polyquaternium 10, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl

pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines quaternized ammonium salt polymers, imidazoline, alkyl pyridinium salts, amines, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

57. (Previously Presented) The method of claim 56, wherein the amine is selected from the group consisting of alkylamines, dialkylamines, alkanolamines, polyethylenepolyamines, N,N-dialkylaminoalkyl acrylates, vinyl pyridine, amine salts, lauryl amine acetate, stearyl amine acetate, alkylpyridinium salt, alkylimidazolium salt, amine oxides, and imide azolinium salts.

58. (Original) The method of claim 55, wherein the cationic surface stabilizer is a nonpolymeric compound selected from the group consisting of benzalkonium chloride, a carbonium compound, a phosphonium compound, an oxonium compound, a halonium compound, a cationic organometallic compound, a quarternary phosphorous compound, a pyridinium compound, an anilinium compound, an ammonium compound, a hydroxylammonium compound, a primary ammonium compound, a secondary ammonium compound, a tertiary ammonium compound, behenalkonium chloride, benzethonium chloride, cetylpyridinium chloride, behentrimonium chloride, lauralkonium chloride, cetalkonium chloride, cetrimonium bromide, cetrimonium chloride, cethylamine hydrofluoride, chlorallylmethenamine chloride (Quaternium-15), distearyldimonium chloride (Quaternium-5), dodecyl dimethyl ethylbenzyl ammonium chloride(Quaternium-14), Quaternium-22, Quaternium-26, Quaternium-18 hectorite, dimethylaminoethylchloride hydrochloride, cysteine hydrochloride, diethanolammonium POE (10) oletyl ether phosphate, diethanolammonium POE (3)oleyl ether phosphate, tallow alkonium chloride, dimethyl dioctadecylammoniumbentonite, stearalkonium chloride, domiphen bromide, denatonium benzoate, myristalkonium chloride, laurtrimonium chloride, ethylenediamine dihydrochloride, guanidine hydrochloride, pyridoxine HCl, iofetamine hydrochloride, meglumine hydrochloride, methylbenzethonium chloride, myrtrimonium bromide, oleyltrimonium chloride, polyquaternium-1, procainehydrochloride, cocobetaine, stearalkonium bentonite,

stearalkoniumhectonite, stearyl trihydroxyethyl propylenediamine dihydrofluoride, tallowtrimonium chloride, and hexadecyltrimethyl ammonium bromide.

59. (Original) The method according to any of claims 53, 55, 56, 57, or 58, wherein the fluticasone composition is bioadhesive.

60. (Previously Presented) A method of treating a subject in need of either symptomatic or prophylactic treatment with a sterile particulate fluticasone composition comprising the step of administering to the subject an effective amount of the sterile particulate fluticasone composition sterilized by passing the composition through a 0.2μm filter, wherein the sterile particulate fluticasone composition comprises particles of fluticasone or a salt thereof and at least one surface stabilizer, wherein the fluticasone particles have an effective average particle size of less than 150 nm.

61. (Previously Presented) The method of claim 60, wherein the effective average particle size of the fluticasone particles is selected from the group consisting of less than 140 nm, less than 130 nm, less than 120 nm, less than 110 nm, less than 100 nm, less than 90 nm, less than 80 nm, less than 70 nm, less than 60 nm, and less than 50 nm.

62.-63. (Cancelled)

64. (Previously Presented) The method of claim 60, wherein the subject has a condition selected from the group consisting of a respiratory related illness, inflammatory airways diseases, obstructive airways diseases, Whipple's disease, AIDS related pneumonia, asthma, emphysema, respiratory distress syndrome, chronic obstructive pulmonary disease, chronic bronchitus, cystic fibrosis, pneumonia, acquired immune deficiency syndrome related respiratory disorders, seasonal rhinitis, perennial rhinitis, seasonal allergic rhinitis, seasonal nonallergic rhinitis, perennial allergic rhinitis, perennial nonallergic rhinitis, and skin conditions treatable with topical corticosteroids.

65. (Original) The method of claim 64, wherein the subject has a condition selected from the group consisting of intrinsic (non-allergic) asthma, extrinsic (allergic) asthma, wheezy-infant syndrome, acute lung injury, acute respiratory distress syndrome, chronic obstructive pulmonary disease, chronic obstructive airways disease, chronic obstructive lung disease, chronic bronchitis, emphysema, bronchiectasis, exacerbation of airways hyperreactivity consequent to other drug therapy, and pneumoconiosis.

66. (Previously Presented) The method of claim 60, wherein the prophylactic efficacy of the treatment is evidenced by one or more characteristics selected from the group consisting of reduced frequency of symptomatic attack, reduced severity of symptomatic attack, improvement in lung function, improved airways hyperreactivity, and a reduced requirement for other symptomatic therapy.

67. (Original) The method of claim 60, wherein the subject is a human.

68. (Cancelled)

69. (Previously Presented) The method of claim 60, wherein the fluticasone has a phase selected from the group consisting of a crystalline phase, an amorphous phase and a semi-crystalline phase.

70. (Previously Presented) The method of claim 60, wherein the sterile particulate fluticasone composition is formulated into a dosage form suitable for administration to a patient, wherein said route of administration is selected from the group consisting of oral, pulmonary, rectal, opthalmic, colonic, parenteral, intracisternal, intravaginal, intraperitoneal, local, buccal, nasal, and topical administration.

71. (Original) The method of claim 60, wherein the fluticasone composition further comprises one or more pharmaceutically acceptable excipients, carriers, or a combination thereof.

72. (Previously Presented) The method of claim 71, wherein the particulate fluticasone is present in the sterile particulate fluticasone composition in an amount selected from the group consisting of from 99.5% to 0.001%, from 95% to 0.1%, and from 90% to 0.5%, by weight, based on the total combined dry weight of the fluticasone and at least one surface stabilizer, not including other excipients.

73. (Previously Presented) The method of claim 71, wherein the at least one surface stabilizer is present in the sterile particulate fluticasone composition in an amount selected from the group consisting of from 0.5% to 99.999%, from 5.0% to 99.9%, and from 10% to 99.5%, by weight, based on the total combined dry weight of the fluticasone and at least one surface stabilizer, not including other excipients.

74. (Previously Presented) The method of claim 60, wherein the sterile particulate fluticasone composition comprises at least two surface stabilizers.

75. (Original) The method of claim 60, wherein the surface stabilizer is selected from the group consisting of an anionic surface stabilizer, a cationic surface stabilizer, a zwitterionic surface stabilizer, and an ionic surface stabilizer.

76. (Previously Presented) The method of claim 75, wherein the at least one surface stabilizer is selected from the group consisting of cetyl pyridinium chloride, gelatin, casein, phosphatides, dextran, glycerol, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oils, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, dodecyl trimethyl ammonium bromide, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, hydroxypropyl celluloses, hypromellose, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hypromellose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol,

polyvinylpyrrolidone, 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde, poloxamers; poloxamines, a charged phospholipid, dioctylsulfosuccinate, dialkylesters of sodium sulfosuccinic acid, sodium lauryl sulfate, alkyl aryl polyether sulfonates, mixtures of sucrose stearate and sucrose distearate, p-isononylphenoxypoly-(glycidol), decanoyl-N-methylglucamide; n-decyl β-D-glucopyranoside; n-decyl β-D-maltopyranoside; n-dodecyl β-D-glucopyranoside; n-dodecyl β-D-maltoside; heptanoyl-N-methylglucamide; n-heptyl-β-D-glucopyranoside; n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside; nonanoyl-N-methylglucamide; n-noyl β-D-glucopyranoside; octanoyl-N-methylglucamide; n-octyl-β-D-glucopyranoside; octyl β-D-thioglucopyranoside; lysozyme, PEG-derivatized phospholipid, PEG-derivatized cholesterol, PEG-derivatized cholesterol, PEG-derivatized vitamin A, PEG-derivatized vitamin E, and random copolymers of vinyl acetate and vinyl pyrrolidone.

77. (Previously Presented) The method of claim 75, wherein the at least one cationic surface stabilizer is selected from the group consisting of a polymer, a biopolymer, a polysaccharide, a cellulosic, an alginate, a nonpolymeric compound, a phospholipid, zwitterionic stabilizers, poly-n-methylpyridinium, anthryul pyridinium chloride, chitosan, polylysine, polyvinylimidazole, polybrene, polymethylmethacrylate trimethylammoniumbromide bromide (PMMTMABr), hexyldesyltrimethylammonium bromide (HDMAB), polyvinylpyrrolidone-2-dimethylaminoethyl methacrylate dimethyl sulfate, 1,2 Dipalmitoyl-sn-Glycero-3-Phosphoethanolamine-N-[Amino(Polyethylene Glycol)2000] (sodium salt), Poly(2-methacryloxyethyl trimethylammonium bromide), poloxamines, lysozyme, alginic acid, carrageenan, and nonionic, high molecular weight, watersoluble poly(ethylene oxide) polymers.

78. (Previously Presented) The method of claim 75, wherein the at least one cationic surface stabilizer is selected from the group consisting of cationic lipids, sulfonium, phosphonium, quarternary ammonium compounds, stearyltrimethylammonium chloride, benzyl-di(2-chloroethyl)ethylammonium bromide, coconut trimethyl ammonium chloride, coconut

trimethyl ammonium bromide, coconut methyl dihydroxyethyl ammonium chloride, coconut methyl dihydroxyethyl ammonium bromide, decyl triethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium chloride, decyl dimethyl hydroxyethyl ammonium bromide, C_{12-15}dimethyl hydroxyethyl ammonium chloride, C_{12-15}dimethyl hydroxyethyl ammonium bromide, coconut dimethyl hydroxyethyl ammonium chloride, coconut dimethyl hydroxyethyl ammonium bromide, myristyl trimethyl ammonium methyl sulphate, lauryl dimethyl benzyl ammonium chloride, lauryl dimethyl benzyl ammonium bromide, lauryl dimethyl (ethenoxy)$_4$ ammonium chloride, lauryl dimethyl (ethenoxy)$_4$ ammonium bromide, N-alkyl (C_{12-18})dimethylbenzyl ammonium chloride, N-alkyl (C_{14-18})dimethyl-benzyl ammonium chloride, N-tetradecylidmethylbenzyl ammonium chloride monohydrate, dimethyl didecyl ammonium chloride, N-alkyl and (C_{12-14}) dimethyl 1-napthylmethyl ammonium chloride, trimethylammonium halide, alkyl-trimethylammonium salts, dialkyl-dimethylammonium salts, lauryl trimethyl ammonium chloride, ethoxylated alkyamidoalkyldialkylammonium salt, an ethoxylated trialkyl ammonium salt, dialkylbenzene dialkylammonium chloride, N-didecyldimethyl ammonium chloride, N-tetradecyldimethylbenzyl ammonium, chloride monohydrate, N-alkyl(C_{12-14}) dimethyl 1-naphthylmethyl ammonium chloride, dodecyldimethylbenzyl ammonium chloride, dialkyl benzenealkyl ammonium chloride, lauryl trimethyl ammonium chloride, alkylbenzyl methyl ammonium chloride, alkyl benzyl dimethyl ammonium bromide, C_{12}, C_{15}, C_{17} trimethyl ammonium bromides, dodecylbenzyl triethyl ammonium chloride, poly-diallyldimethylammonium chloride (DADMAC), dimethyl ammonium chlorides, alkyldimethylammonium halogenides, tricetyl methyl ammonium chloride, decyltrimethylammonium bromide, dodecyltriethylammonium bromide, tetradecyltrimethylammonium bromide, methyl trioctylammonium chloride, polyquaternium 10, tetrabutylammonium bromide, benzyl trimethylammonium bromide, choline esters, benzalkonium chloride, stearalkonium chloride compounds, cetyl pyridinium bromide, cetyl pyridinium chloride, halide salts of quaternized polyoxyethylalkylamines, quaternized

ammonium salt polymers, imidazoline, alkyl pyridinium salts, amines, protonated quaternary acrylamides, methylated quaternary polymers, and cationic guar.

79. (Previously Presented) The method of claim 78, wherein the amine is selected from the group consisting of alkylamines, dialkylamines, alkanolamines, polyethylenepolyamines, N,N-dialkylaminoalkyl acrylates, vinyl pyridine, amine salts, lauryl amine acetate, stearyl amine acetate, alkylpyridinium salt, alkylimidazolium salt, amine oxides, and imide azolinium salts.

80. (Original) The method of claim 77, wherein the cationic surface stabilizer is a nonpolymeric compound selected from the group consisting of benzalkonium chloride, a carbonium compound, a phosphonium compound, an oxonium compound, a halonium compound, a cationic organometallic compound, a quarternary phosphorous compound, a pyridinium compound, an anilinium compound, an ammonium compound, a hydroxylammonium compound, a primary ammonium compound, a secondary ammonium compound, a tertiary ammonium compound, behenalkonium chloride, benzethonium chloride, cetylpyridinium chloride, behentrimonium chloride, lauralkonium chloride, cetalkonium chloride, cetrimonium bromide, cetrimonium chloride, cethylamine hydrofluoride, chlorallylmethenamine chloride (Quaternium-15), distearyldimonium chloride (Quaternium-5), dodecyl dimethyl ethylbenzyl ammonium chloride(Quaternium-14), Quaternium-22, Quaternium-26, Quaternium-18 hectorite, dimethylaminoethylchloride hydrochloride, cysteine hydrochloride, diethanolammonium POE (10) oletyl ether phosphate, diethanolammonium POE (3)oleyl ether phosphate, tallow alkonium chloride, dimethyl dioctadecylammoniumbentonite, stearalkonium chloride, domiphen bromide, denatonium benzoate, myristalkonium chloride, laurtrimonium chloride, ethylenediamine dihydrochloride, guanidine hydrochloride, pyridoxine HCl, iofetamine hydrochloride, meglumine hydrochloride, methylbenzethonium chloride, myrtrimonium bromide, oleyltrimonium chloride, polyquaternium-1, procainehydrochloride, cocobetaine, stearalkonium bentonite,

stearalkoniumhectonite, stearyl trihydroxyethyl propylenediamine dihydrofluoride, tallowtrimonium chloride, and hexadecyltrimethyl ammonium bromide.

81. (Original) The method according to any of claims 75, 77, 78, 79, or 80, wherein the composition is bioadhesive.

82.–99. (Cancelled)

APPENDIX B: EVIDENCE

1. U.S. Patent No. 5,747,001 to Wiedmann et al.;

2. U.S. Patent No. 6,241,969 to Saidi et al.;

3. PCT Publication No. WO 96/25918 by Wood et al.;

4. U.S. Patent Application Publication No. 2003/0073676 by Biggadike et al.

APPENDIX B: EVIDENCE

1.
U.S. Patent No. 5,747,001
to Wiedmann et al.

US005747001A

United States Patent [19]

Wiedmann et al.

[11] Patent Number: 5,747,001

[45] Date of Patent: May 5, 1998

[54] **AEROSOLS CONTAINING BECLOMETHAZONE NANOPARTICLE DISPERSIONS**

[75] Inventors: **Timothy S. Wiedmann**, Minneapolis, Minn.; **Ray W. Wood**, Ft. Washington; **Lan DeCastro**, West Chester, both of Pa.

[73] Assignee: **NanoSystems, L.L.C.**, King of Prussia, Pa.

[21] Appl. No.: **393,973**

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[51] **Int. Cl.**[6] **A61K 9/12**

[52] **U.S. Cl.** **424/45**; 424/46; 424/489

[58] **Field of Search** 424/45, 46, 489; 514/826

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,810,488	3/1989	Jinks	424/45
5,145,684	9/1992	Liversidge et al.	424/489
5,225,183	7/1993	Purewal et al.	424/45

FOREIGN PATENT DOCUMENTS

03153	2/1994	WIPO .

Primary Examiner—Raj Bawa

Attorney, Agent, or Firm—McDermott, Will & Emery

[57] **ABSTRACT**

There is disclosed an aerosol comprising droplets of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble beclomethazone particles having a surface modifier on the surface thereof. There is also disclosed a method for making the aerosol and methods for treatment using the aerosol.

10 Claims, No Drawings

AEROSOLS CONTAINING BECLOMETHAZONE NANOPARTICLE DISPERSIONS

FIELD OF THE INVENTION

The present invention is directed to the field of nanoparticles and particularly beclomethazone containing nanoparticles in an aerosol form.

BACKGROUND OF THE INVENTION

Delivery of therapeutic agent to the respiratory tract is important for both local and systemic treatment of disease. With the conventional techniques, delivery of agents to the lung is extremely inefficient. Attempts to develop respirable aqueous suspensions of poorly soluble compounds have been unsuccessful. Micronized therapeutic agents suspended in aqueous media are too large to be delivered by aerosolized aqueous droplets. With conventional processes, it is estimated that only about 10 to 20% of the agent reaches the lung. Specifically, there is loss to the device used to deliver the agent, loss to the mouth and throat and with exhalation. These losses lead to variability in therapeutic agent levels and poor therapeutic control. In addition, deposition of the agent to the mouth and throat can lead to systemic absorption and undesirable side effects.

The efficiency of respiratory drug delivery is largely determined by the particle size distribution. Large particles (greater than 10 μm) are primarily deposited on the back of the throat. Greater than 60% of the particles with sizes between 1 and 10 μm pass with the air stream into the upper bronchial region of the lung where most are deposited. With particles less than about 1 μm, essentially all of the particles enter the lungs and pass into the peripheral alveolar region; however, about 70% are exhaled and therefore are lost.

In addition to deposition, the relative rate of absorption and rate of clearance of the therapeutic agent must be considered for determining the amount of therapeutic agent that reaches the site of action. Since 99.99% of the available area is located in the peripheral alveoli, rapid absorption can be realized with delivery of the particles to the periphery. For clearance, there is also differences between the central and peripheral regions of the lung. The peripheral alveolar region does not have ciliated cells but relies on macrophage engulfment for particle clearance. This much slower process can significantly extend the time during which the particles reside in the lung thereby enhancing the therapeutic or diagnostic effect. In contrast, particles deposited in the upper respiratory tract are rapidly cleared by mucociliary escalator. That is, the particles are trapped in the mucous blanket coating the lung surface and are transported to the throat. Hence, this material is either swallowed or removed by coughing.

While it has long been known that smaller droplets of an aerosol reach deeper into the respiratory system (*Current Concepts in the Pharmaceutical Sciences: Dosage and Bioavailability*, J. Swarbrick Ed., Lea and Febiger, Philadelphia, Pa., 1973, pp. 97–148) these have largely been of theoretical interest. Simply knowing that smaller droplets of aersol can be delivered deeper into the respiratory system does not solve the problem of incorporating sufficient therapeutic agent into the aerosol to be efficient, particularly where the therapeutic agent is only slightly soluble in the liquid for the aerosol.

Nanoparticles, described in U.S. Pat. No. 5,145,684, are particles consisting of a poorly soluble therapeutic or diagnostic agent onto which are adsorbed a non-crosslinked surface modifier, and which have an average particle size of less than about 400 nanometers (nm). However, no mention is made of attempts to nebulize (aerosolize or atomize are equivalent terms for the purpose of this disclosure) these compositions and it is not apparent that nebulizing these composition would provide useful aerosols or that there would be any advantage for doing so.

Beclomethazone dipropionate monohydrate is an antiinflamatory steroid that is commercially available in the form of a nasal spray. According to the Physicians' Desk Reference®, it is sparingly soluble and when given by nasal inhalation in the form of an aqueous or aerosolized suspension, the drug is deposited primarily in the nasal passages. A portion of the drug is swallowed. Thus, delivery of beclomethazone is prone to all of the problems known for aerosolized suspensions of slightly soluble drugs mentioned above.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided an aerosol comprising droplets of an aqueous dispersion of nanoparticles, said nanoparticles comprising beclomethasone having a surface modifier on the surface thereof.

In another aspect of the invention, there is provided a method for forming an aerosol of a nanoparticle dispersion, said nanoparticles comprising beclomethasone particles having a surface modifier on the surface thereof, said method comprising the steps of:

a) providing a suspension of said nanoparticles;

b) nebulizing said suspension so as to form an aerosol.

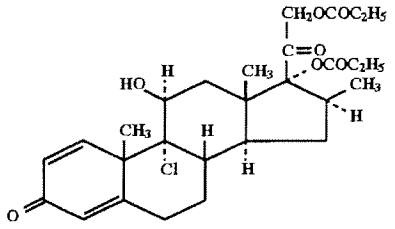
In yet another aspect of the invention, there is provided a method of treating a mammal comprising the steps of:

a) forming an aerosol of an aqueous dispersion of nanoparticles, said nanoparticles comprising beclomethasone having a surface modifier on the surface thereof;

b) administering said aerosol to the respiratory system of said mammal.

DETAILED DESCRIPTION OF THE INVENTION

Beclomethazone dipropionate has the structural formula:



It is a white powder with a molecular weight of 521.25; and is very slightly soluble in water. As used herein, the term beclomethazone means free beclomethazone; its various mono- and diesters. Specifically included is the preferred form, beclomethazone dipropionate and its monohydrate

The compositions of the invention are aerosols. Aerosols can be defined for the present purpose as colloidal systems consisting of very finely divided liquid droplets dispersed in and surounded by a gas. The droplets in the aerosols typically have a size less than about 50 microns in diameter although droplets of a much smaller size are possible.

The aerosols of the present invention are particularly useful in the treatment of respiratory related illnesses.

Beclomethazone is particularly useful in the treatment of seasonal or perennial rhinitis and is also indicated for the relief of the symptoms of seasonal or perennial allergic an nonallergic (vasomotor) rhinitis.

The aerosols of the invention are made by nebulizing the nanoparticle containing solution using a variety of known nebulizing techniques. Perhaps the simplest of systems is the "two-phase" system which consists of a solution or a suspension of active ingredient, in the present case, a nanoparticle containing a beclomethazone, in a liquid propellent. Both liquid and vapor phases are present in a pressurized container and when a valve on the container is opened, liquid propellent containing the nanoparticle dispersion is released. Depending on the nature of the ingredients and the nature of the valve mechanism, a fine aersol mist or aersol wet spray is produced.

There are a variety of nebulisers that are available to produce the aerosols of the invention including small volume nebulizers. Compressor driven nebulizers incorporate jet technology and use compressed air to generate the aersol. Commercially available devices are available from Healthdyne Technologies Inc; Invacare Inc.; Mountain Medical Equipment Inc.; Pari Respiratory Inc.; Mada Mediacal Inc.; Puritan-Bennet; Schuco Inc.; Omron Healthcare Inc.; DeVilbiss Health Care Inc; and Hospitak Inc.

Ultrasonic nebulizers deliver high medication output and are used by patients suffering from severe asthma, or other severe respiratory related illnesses.

Surface Modifiers

Suitable surface modifiers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products and surfactants. Preferred surface modifiers include nonionic and ionic surfactants.

Representative examples of surface modifiers include gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens™, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxy propylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidone (PVP). Most of these surface modifiers are known pharmaceutical excipients and are described in detail in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986.

Particularly preferred surface modifiers include polyvinylpyrrolidone, tyloxapol, poloxamers such as Pluronic™ F68 and F108, which are block copolymer of ethylene oxide and propylene oxide, and polyxamines such as Tetronic™ 908 (also known as Poloxamine™ 908), which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine, available from BASF, dextran, lecithin, dialkylesters of sodium sulfosuccinic acid, such as Aerosol OT™, which is a dioctyl ester of sodium sulfosuccinic acid, available from American Cyanimid, Duponol™ P, which is a sodium lauryl sulfate, available from DuPont, Triton™ X-200, which is an alkyl aryl polyether sulfonate, available from Rohn and Haas, Tween™ 20 and Tween™ 80, which are polyoxyethylene sorbitan fatty acid esters, available from ICI Specialty Chemicals; Carbowax™ 3550 and 934, which are polyethylene glycols available from Union Carbide; Crodesta™ F-110, which is a mixture of sucrose stearate and sucrose distearate, available from Croda Inc., Crodesta™ SL-40, which is available from Croda, Inc., and SA90HCO, which is $C_{18}H_{37}$—$CH_2(CON(CH_3)CH_2(CHOH)_4CH_2OH)_2$. Surface modifiers which have been found to be particularly useful include Tetronic™ 908, the Tweens™, Pluronic™ F-68 and polyvinylpyrrolidone. Other useful surface modifiers include:

decanoyl-N-methylglucamide;

n-decyl β-D-glucopyranoside;

n-decyl β-D-maltopyranoside;

n-dodecyl β-D-glucopyranoside;

n-dodecyl β-D-maltoside;

heptanoyl-N-methylglucamide;

n-heptyl-β-D-glucopyranoside;

n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside;

nonanoyl-N-methylglucamide;

n-noyl β-D-glucopyranoside;

octanoyl-N-methylglucamide;

n-octyl-β-D-glucopyranoside;

octyl β-D-thioglucopyranoside; and the like.

Another useful surface modifier is tyloxapol (a nonionic liquid polymer of the alkyl aryl polyether alcohol type; also known as superinone or triton). This surface modifier is commercially available and/or can be prepared by techniques known in the art.

Another preferred surface modifier is p-isononylphenoxypoly(glycidol) also known as Olin-10G™ or Surfactant 10-G, is commercially available as 10G™ from Olin Chemicals, Stamford, Conn.

Non-Ionic Surface Modifiers

Preferred surface modifiers can be selected from known non-ionic surfactants, including the poloxamines such as Tetronic™ 908 (also known as Poloxamine™ 908), which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine, available from BASF, or Tetronic™ 1508 (T-1508), or a polymer of the alkyl aryl polyether alcohol type, such as tyloxapol.

The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two or more surface modifiers can be used in combination.

Tyloxapol

Tyloxapol (4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde) is a preferred surface modifier and is a nonionic liquid polymer of the alkyl aryl polyether alcohol type. Tyloxapol, also known as "Superinone", is disclosed as useful as a nonionic surface active agent in a lung surfactant composition in U.S. Pat. No. 4,826,821 and as a stabilizing agent for 2-dimethylaminoethyl 4-n-butylaminobenzoate in U.S. Pat. No. 3,272,700.

Tyloxapol may be associated with the nanoparticles and may function as a surface modifier, as a stabilizer, and/or as a dispersant. Alternatively, the tyloxapol may serve other purposes. Tyloxapol may serve all three functions. The tyloxapol may serve as a stabilizer and/or a dispersant, whereas another compound acts as a surface modifier.

Auxiliary Surface Modifiers

Particularly preferred auxiliary surface modifiers are those which impart resistance to particle aggregation during sterilization and include dioctylsulfosuccinate (DOSS), polyethylene glycol, glycerol, sodium dodecyl sulfate, dodecyl trimethyl, ammonium bromide and a charged phospholipid such as dimyristoyl phophatidyl glycerol. The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two or more surface modifiers can be used in combination.

Block Copolymer Surface Modifiers

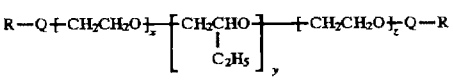
One preferred surface modifier is a block copolymer linked to at least one anionic group. The polymers contain at least one, and preferably two, three, four or more anionic groups per molecule. Preferred anionic groups include sulfate, sulfonate, phosphonate, phosphate and carboxylate groups. The anionic groups are covalently attached to the nonionic block copolymer. The nonionic sulfated polymeric surfactant has a molecular weight of 1,000–50,000, preferably 2,000–40,000 and more preferably 3,000–30,000. In preferred embodiments, the polymer comprises at least about 50%, and more preferably, at least about 60% by weight of hydrophilic units, e.g., alkylene oxide units. The reason for this is that the presence of a major weight proportion of hydrophilic units confers aqueous solubility to the polymer.

A preferred class of block copolymer useful as surface modifiers herein includes sulfated block copolymers of ethylene oxide and propylene oxide. These block copolymer in an unsulfated form are commercially available as Pluronics™. Specific examples of the unsulfated block copolymer include F68, F108 and F127.

Another preferred class of block copolymer useful herein include tetrafunctional block copolymer derived from sequential addition of ethylene oxide and propylene oxide to ethylene diamine. These polymers, in an unsulfated form, are commercially available as Tetronics™.

Another preferred class of surface modifiers contain at least one polyethylene oxide (PEO) block as the hydrophilic portion of the molecule and at least one polybutylene oxide (PBO) block as the hydrophobic portion. Particularly preferred surface modifiers of this class are diblock, triblock, and higher block copolymer of ethylene oxide and butylene oxide, such as are represented, for example, by the following structural formula: -(PEO)-(PBO)-; -(PEO)-(PBO)-(PEO)-; and -(PEO)-(PBO)-(PEO)-(PBO)-. The block copolymer useful herein are known compounds and/or can be readily prepared by techniques well known in the art.

Highly preferred surface modifiers include triblock copolymer of the structure -(PEO)-(PBO)-(PEO)- having molecular weights of 3800 and 5000 which are commercially available from Dow Chemical, Midland, Mich., and are referred to as B20-3800 and B20-5000. These surface modifiers contain about 80% by weight PEO. In a preferred embodiment, the surface modifier is a triblock polymer having the structure:



Q is an anionic group

wherein R is H or a metal cation such as Na^+, K^+ and the like, x is 15–700, y is 5–200 and z is 15–700.

Grinding

The described particles can be prepared in a method comprising the steps of dispersing beclomethazone in a liquid dispersion medium and applying mechanical means in the presence of grinding media to reduce the particle size of the beclomethazone to an effective average particle size of less than about 400 nm. The particles can be reduced in size in the presence of a surface modifier. Alternatively, the particles can be contacted with a surface modifier after, attrition.

The beclomethazone is obtained commercially and/or prepared by techniques known in the art in a conventional coarse form. It is preferred, but not essential, that the particle size of the coarse beclomethazone selected be less than about 100 μm as determined by sieve analysis. If the coarse particle size of the beclomethazone is greater than about 100 μm, then it is preferred that the particles of the beclomethazone be reduced in size to less than 100 μm using a conventional milling method such as airjet or fragmentation milling.

The coarse beclomethazone selected can then be added to a liquid medium in which it is essentially insoluble to form a premix. The concentration of the beclomethazone in the liquid medium can vary from about 0.1–60%, and preferably is from 5–30% (w/w). It is preferred, but not essential, that the surface modifier be present in the premix. The concentration of the surface modifier can vary from about 0.1 to about 90%, and preferably is 1–75%, more preferably 20–60%, by weight based on the total combined weight of the beclomethazone and surface modifier. The apparent viscosity of the premix suspension is preferably less than about 1000 centipoise.

The premix can be used directly by subjecting it to mechanical means to reduce the average particle size in the dispersion to less than 400 nm. It is preferred that the premix be used directly when a ball mill is used for attrition. Alternatively, the beclomethazone and, optionally, the surface modifier, can be dispersed in the liquid medium using suitable agitation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition.

The mechanical means applied to reduce the particle size of the beclomethazone conveniently can take the form of a dispersion mill. Suitable dispersion mills include a ball mill, an attritor mill, a vibratory mill, and media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the intended result, i.e., the desired reduction in particle size. For media milling, the apparent viscosity of the premix preferably is from about 100 to about 1000 centipoise. For ball milling, the apparent viscosity of the premix preferably is from about 1 up to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle fragmentation and media erosion.

Preparation Conditions

The attrition time can vary widely and depends primarily upon the particular mechanical means and processing conditions selected. For ball mills, processing times of up to five days or longer may be required. On the other hand, processing times of less than 1 day (residence times of one minute up to several hours) have provided the desired results using a high shear media mill.

The particles must be reduced in size at a temperature which does not significantly degrade the beclomethazone. Processing temperatures of less than about 30°–40° C. are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. The method is conveniently carried out under conditions of

ambient temperature and at processing pressures which are safe and effective for the milling process. For example, ambient processing pressures are typical of ball mills, attritor mills and vibratory mills. Control of the temperature, e.g., by jacketing or immersion of the milling chamber in ice water are contemplated. Processing pressures from about 1 psi (0.07 kg/cm^2) up to about 50 psi (3.5 kg/cm^2) are contemplated. Processing pressures from about 10 psi (0.7 kg/cm^2) to about 20 psi (1.4 kg/cm^2) are typical.

The surface modifier, if it was not present in the premix, must be added to the dispersion after attrition in an amount as described for the premix above. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20–80 kHz for a time of about 1 to 120 seconds.

After attrition is completed, the grinding media is separated from the milled particulate product (in either a dry or liquid dispersion form) using conventional separation techniques, such as by filtration, sieving through a mesh screen, and the like.

Grinding Media

The grinding media for the particle size reduction step can be selected from rigid media preferably spherical or particulate in form having an average size less than about 3 mm and, more preferably, less than about 1 mm. Such media desirably can provide the particles with shorter processing times and impart less wear to the milling equipment. The selection of material for the grinding media is not believed to be critical. We have found that zirconium oxide, such as 95% ZrO_2 stabilized with magnesia, zirconium silicate, and glass grinding media provide particles having levels of contamination which are believed to be acceptable for the preparation of pharmaceutical compositions. However, other media, such as stainless steel, titania, alumina, and 95% ZrO_2 stabilized with yttrium, are expected to be useful. Preferred media have a density greater than about 3 g/cm^3.

Polymeric Grinding Media

The grinding media can comprise particles, preferably substantially spherical in shape, e.g., beads, consisting essentially of polymeric resin. Alternatively, the grinding media can comprise particles comprising a core having a coating of the polymeric resin adhered thereon.

In general, polymeric resins suitable for use herein are chemically and physically inert, substantially free of metals, solvent and monomers, and of sufficient hardness and friability to enable them to avoid being chipped or crushed during grinding. Suitable polymeric resins include crosslinked polystyrenes, such as polystyrene crosslinked with divinylbenzene, styrene copolymer, polycarbonates, polyacetals, such as Delrin™, vinyl chloride polymers and copolymer, polyurethanes, polyamides, poly (tetrafluoroethylenes), e.g., Teflon™, and other fluoropolymers, high density polyethylenes, polypropylenes, cellulose ethers and esters such as cellulose acetate, polyhydroxymethacrylate, polyhydroxyethyl acrylate, silicone containing polymers such as polysiloxanes and the like. The polymer can be biodegradable. Exemplary biodegradable polymers include poly(lactides), poly (glycolide) copolymer of lactides and glycolide, polyanhydrides, poly(hydroxyethyl methacylate), poly (imino carbonates), poly(N-acylhydroxyproline)esters, poly (N-palmitoyl hydroxyproline) esters, ethylene-vinyl acetate copolymer, poly(orthoesters), poly(caprolactones), and poly (phosphazenes). In the case of biodegradable polymers, contamination from the media itself advantageously can metabolize in vivo into biologically acceptable products which can be eliminated from the body.

The polymeric resin can have a density from 0.8 to 3.0 g/cm^3. Higher density resins are preferred inasmuch as it is believed that these provide more efficient particle size reduction.

The media can range in size from about 0.1 to 3 mm. For fine grinding, the particles preferably are from 0.2 to 2 mm, more preferably, 0.25 to 1 mm in size.

In a particularly preferred method, a beclomethazone is prepared in the form of submicron particles by grinding the agent in the presence of a grinding media having a mean particle size of less than about 75 microns.

The core material of the grinding media preferably can be selected from materials known to be useful as grinding media when fabricated as spheres or particles. Suitable core materials include zirconium oxides (such as 95% zirconium oxide stabilized with magnesia or yttrium), zirconium silicate, glass, stainless steel, titania, alumina, ferrite and the like. Preferred core materials have a density greater than about 2.5 g/cm^3. The selection of high density core materials is believed to facilitate efficient particle size reduction.

Useful thicknesses of the polymer coating on the core are believed to range from about 1 to about 500 microns, although other thicknesses outside this range may be useful in some applications. The thickness of the polymer coating preferably is less than the diameter of the core.

The cores can be coated with the polymeric resin by techniques known in the art. Suitable techniques include spray coating, fluidized bed coating, and melt coating. Adhesion promoting or tie layers can optionally be provided to improve the adhesion between the core material and the resin coating. The adhesion of the polymer coating to the core material can be enhanced by treating the core material to adhesion promoting procedures, such as roughening of the core surface, corona discharge treatment, and the like.

Continuous Grinding

In a preferred grinding process, the particles are made continuously rather than in a batch mode. The continuous method comprises the steps of continuously introducing the beclomethazone and rigid grinding media into a milling chamber, contacting the agent with the grinding media while in the chamber to reduce the particle size of the agent, continuously removing the agent and the grinding media from the milling chamber, and thereafter separating the agent from the grinding media.

The beclomethazone and the grinding media are continuously removed from the milling chamber. Thereafter, the grinding media is separated from the milled particulate agent (in either a dry or liquid dispersion form) using conventional separation techniques, in a secondary process such as by simple filtration, sieving through a mesh filter or screen, and the like. Other separation techniques such as centrifugation may also be employed.

In a preferred embodiment, the agent and grinding media are recirculated through the milling chamber. Examples of suitable means to effect such recirculation include conventional pumps such as peristaltic pumps, diaphragm pumps, piston pumps, centrifugal pumps and other positive displacement pumps which do not use sufficiently close tolerances to damage the grinding media. Peristaltic pumps are generally preferred.

Another variation of the continuous process includes the use of mixed media sizes. For example, larger media may be employed in a conventional manner where such media is restricted to the milling chamber. Smaller grinding media may be continuously recirculated through the system and

permitted to pass through the agitated bed of larger grinding media. In this embodiment, the smaller media is preferably between about 1 and 300 mm in mean particle size and the larger grinding media is between about 300 and 1000 mm in mean particle size.

Precipitation Method

Another method of forming the desired nanoparticle dispersion is by microprecipitation. This is a method of preparing stable dispersions of beclomethazone in the presence of a surface modifying and colloid stability enhancing surface active agent free of trace of any toxic solvents or solubilized heavy metal inpurities by the following procedural steps:

1. Dissolving the beclomethazone in aqueous base with stirring,
2. Adding above #1 formulation with stirring to a surface active surfactant (or surface modifiers) solution to form a clear solution, and,
3. Neutralizing above formulation #2 with stirring with an appropriate acid solution. The procedure can be followed by:
4. Removal of formed salt by dialysis or diafiltration and
5. Concentration of dispersion by conventional means.

This microprecipitation process produces dispersion of beclomethazone with Z-average particle diameter less than 400 nm (as measured by photon correlation spectroscopy) that are stable in particle size upon keeping under room temperature or refrigerated conditions. Such dispersions also demonstrate limited particle size growth upon autoclave-decontamination conditions used for standard blood-pool pharmaceutical agents.

Step 3 can be carried out in semicontinuous, continuous batch, or continuous methods at constant flow rates of the reacting components in computer-controlled reactors or in tubular reactors where reaction pH can be kept constant using pH-stat systems. Advantages of such modifications are that they provide cheaper manufacturing procedures for large-scale production of nanoparticulate dispersion systems.

Additional surface modifier may be added to the dispersion after precipitation. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20–80 kHz for a time of about 1 to 120 seconds.

In a preferred embodiment, the above procedure is followed with step 4 which comprises removing the formed salts by diafiltration or dialysis. This is done in the case of dialysis by standard dialysis equipment and by diafiltration using standard diafiltration equipment known in the art. Preferably, the final step is concentration to a desired concentration of the agent dispersion. This is done either by diafiltration or evaporation using standard equipment known in this art.

An advantage of microprecipitation is that unlike milled dispersion, the final product is free of heavy metal contaminants arising from the milling media that must be removed due to their toxicity before product is formulated.

A further advantage of the microprecipitation method is that unlike solvent precipitation, the final product is free of any trace of trace solvents that may be toxic and must be removed by expensive treatments prior to final product formulation.

In another preferred embodiment of the microprecipitation process, a crystal growth modifier is used. A crystal growth modifier is defined as a compound that in the co-precipitation process incorporates into the crystal structure of the microprecipitated crystals of the beclomethazone, thereby hindering growth or enlargement of the microcrystalline precipitate, by the so called Ostwald ripening process. A crystal growth modifier (or a CGM) is a chemical that is at least 75% identical in chemical structure to the beclomethazone. By "identical" is meant that the structures are identical atom for atom and their connectivity. Structural identity is characterized as having 75% of the chemical structure, on a molecular weight basis, identical to the beclomethazone. The remaining 25% of the structure may be absent or replaced by different chemical structure in the CGM. The crystal growth modifier is dissolved in step #1 with the beclomethazone.

Particle Size

As used herein, particle size refers to a number average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art, such as sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. When photon correlation spectroscopy (PCS) is used as the method of particle sizing the average particle diameter is the Z-average particle diameter known to those skilled in the art. By "an effective average particle size of less than about 400 nm" it is meant that at least 90% of the particles have a weight average particle size of less than about 400 nm when measured by the above-noted techniques. In preferred embodiments, the effective average particle size is less than about 300 nm and more preferrably less than about 250 nm. In some embodiments, an effective average particle size of less than about 100 nm has been achieved. With reference to the effective average particle size, it is preferred that at least 95% and, more preferably, at least 99% of the particles have a particle size less than the effective average, e.g., 400 nm. In particularly preferred embodiments, essentially all of the particles have a size less than 400 nm. In some embodiments, essentially all of the particles have a size less than 250 nm.

Ratios

The relative amount of beclomethazone and surface modifier can vary widely and the optimal amount of the surface modifier can depend on surface modifier selected, the critical micelle concentration of the surface modifier if it forms micelles, the hydrophilic lipophilic balance (HLB) of the stabilizer, the melting point of the stabilizer, its water solubility, the surface tension of water solutions of the stabilizer, etc. The surface modifier preferably is present in an amount of about 0.1–10 mg per square meter surface area of the beclomethazone. The surface modifier can be present in an amount of 0.1–90%, preferably 20–60% by weight based on the total weight of the dry particle.

The following examples are presented for a further understanding of the invention.

EXAMPLE 1

Materials

Beclomethasone diproprionate (BDP) and polyvinyl alcohol (PVA) were obtained from Sigma Chemical Co. (St. Louis, Mo.) and used as received. All other chemicals were analytical/reagent grade or better.

Nanoparticle Preparation and Characterization

Nanoparticles were prepared by media milling a suspension of 5% beclomethasone diproprionate in an aqueous solutions of PVA. Thus, the PVA was the surface modifier. The resulting particle size distribution was determined by dynamic light scattering. The particle size distribution was periodically monitored throughout the course of the study.

Nebulization

A gas cylinder of compressed air was used as the source, which was equipped with a pressure regulator. Oxygen connecting tubing joined from the regulator to the Puritan-Bennet Raindrop nebulizer (Lenexa, KA). One exit port of the T-connector of the nebulizer was blocked with a #2 rubber stopper. The other exit port was fitted with Tygon tubing (½" id). This in turn led initially to a calibrated flow meter from which the flow rate was set before each experiment. After calibration, the gas flow was stopped by shutting off the main cylinder valve. The flow meter was removed, and the nebulizer was connected to a Y-tube with 24/40 joints by tubing (½" id. 6" length). The Y-tube was connected to the cascade impactor (Andersen Mark I, Andersen Samplers Ind. Atlanta, Ga.) by a constructed stainless steel adapter consisting of a tapered side that fit within the 24/40 ground glass joint and a cylindrical section with rubber o-ring gasket that fit into the top of the cascade impactor. The air flow rate through the impactor was drawn by a vacuum pump and regulated by a calibrated flow meter to the recommended 28.3 L/min.

Preliminary studies indicated that pressures between 20 and 40 psig had little effect on either the performance of the nebulizer or the resulting aerosol size distribution. Thus, the pressure was kept constant at 40 psig. Studies of the effect of flow rate on nebulizer performance and aerosol size distribution were also conducted. As the flow rate was decreased from 5 to 2 L/min, aerosol particles had progressively larger mean aerodynamic diameter. At a flow rate 8 L/min, there was excessive foaming. Thus, all studies were conducted at a flow rate of 6 L/min.

Suspension and Nanoparticle Nebulization

Formulations for nebulization consisted of a 0.2% beclomethasone diproprionate dispersions with PVA. The nebulizers contained either a volume of 2 mL or 6 mL. Two concentrations of PVA were used which were prepared by diluting the original 5% (w/v) nanoparticle dispersion with a PVA solution having the same PVA concentration as the original dispersion concentration or with water. The nebulizer was filled, and aliquots of the solution were taken for subsequent determination of drug concentration. The weight was also determined. The nebulization process was initiated by opening the valve on the main gas cylinder, and the length of time until foaming or sputtering of the nebulizer was determined, and additional aliquots were taken for analysis. The fraction of mass exiting the nebulizer was calculated from the weight difference of the nebulizer before and after nebulization. This was coupled with the time required for nebulization of the dispersion to yield the mass output rate in terms of the milliliters of dispersion nebulized/unit time and the nebulizer output in terms of the volume of dispersion nebulized/liter of air were determined.

Aliquots taken from the nebulizer were diluted with 50% (v/v) ethanol in water, and the absorbance determined at 240 nm. With measurement of the absorbance of appropriate standards, the concentration of BDP was calculated. From the masses of the nebulizer before and after nebulization and the BDP concentrations, the fraction of BDP remaining in the nebulizer was calculated. The mass of BDP collected on the cascade impactor and the aerosol particle size distribution was determined by extracting the impactor stages with 10 mL of the ethanol/water solution. Aliquots were taken and the absorbances and subsequent concentration were determined. The mass median aerodynamic diameter and geometric standard deviation of the particle distribution was obtained by plotting the cumulative mass on the stages of the impactor as a function of the log of the cut-off diameter. With the cumulative mass determined from the cascade impactor and the initial amount of BDP placed in the nebulizer, the fraction of BDP reaching the impactor was calculated.

To assess the fractionation of the dispersion, the nanoparticles and suspensions were diluted with PVA solutions containing 0.1% sodium fluorescein. Nebulization was conducted as described above. Since fluorescein has significant absorbance at both 490 and 240 nm while BDP has absorbance only at 240 nm, the absorbance of the diluted aliquots was determined at these two wavelengths. The concentration of fluorescein was determined from the absorbance at 490 nm and the measured absorptivity. In determining the concentrations of BDP, the contribution from the absorbance of fluorescein at 240 nm was subtracted based on the absorbance determined at 490 and the correction for the differences in the absorptivity at these two wavelengths.

Scanning Electron Microscopy

SEM was performed on nanoparticles after nebulization. Two dispersions were prepared containing 0.1 and 2.5% surfactant. These were placed in the nebulizer and 2 cm rectangular glass microscope slides were placed on every stage of the impactor. The glass slides were removed and sputtered with platinum. Micrographs were obtained with a JEOL 840-II ElectroScan Environmental ESEM (Peabody, Mass.).

RESULTS

Nanoparticles of beclomethasone diproprionate in 2.5% polyvinyl alcohol had a particle size distribution of 0.26±0.13 μm. This size remained constant throughout the course of the study; neither was there any evidence of chemical instability. In addition, particle size of the diluted dispersions remained constant for at least the duration of the experiment.

For nebulization, four formulations were tested. These are listed in Table I. The first was a suspension of raw drug substance BDP in 2.5% surfactant with a volume of 2 mL. The second was composed of a dispersion of nanoparticles thereby allowing direct comparison to the suspension formulation. The third was also a colloidal dispersion, but the surfactant concentration was smaller at 0.1%. The fourth was similar to the third but contained a larger volume of 6 mL.

In Table II, the results from the nebulization of the four formulations were given. The second column provides the mass output rate which was the rate at which the total mass of the dispersion exists the nebulizer. Formulations I and II are similar as were formulations III and IV. The difference between these two sets of formulations is that I and II had a surfactant concentration of 2.5%, whereas III and IV had a surfactant concentration of 0.1%.

The third column reflects the total mass fraction of dispersion remaining in the nebulizer. The fraction of mass remaining was between 0.27 and 0.69 indicating considerable amount of material remained in the nebulizer. In addition, formulations I, II and III were similar, but formulation IV had a significantly lower mass fraction remaining in the nebulizer. Formulation IV is distinct from the others in that it contained an initial volume of 6 mL.

In the next column, the fraction of BDP remaining in the nebulizer is given. These fractions ranged from 0.29 to 0.89. In comparing the fractions remaining, formulation I, which contained the suspension, had about 90% of BDP remain in the nebulizer. In contrast, formulation III which contained 0.1% surfactant, had a significantly lower fraction of BDP

remain in the nebulizer. An even more dramatic drop in fraction remaining was observed with formulation IV which had a low surfactant concentration as well as a larger volume.

It is also noteworthy to compare the fraction of BDP remaining relative to the fraction of total mass remaining in the nebulizer. With formulation I, there was a significantly greater fraction of BDP relative to the total mass remaining. Numerically this is also true for formulation II: however, there was more variability in these measurements which had no statistical difference in the fractions remaining. In formulations III and IV, there was no difference.

The fraction of BDP reaching the nebulizer is also given in Table II. It is seen that only about 7% of the BDP presented as a suspension or raw drug substance reaches the impactor. In comparison, the use of nanoparticles led to a significantly higher fraction reaching the impactor. These ranged from 0.17 to over 0.34. In formulations II and III which contained 2 mL of dispersion, about 18% of BDP reached the impactor. In the large volume formulation IV, almost 35% of BDP reached the impactor.

Finally, it is evident that the amount of BDP that was originally placed in the nebulizer should equal the amount of BDP remaining in the nebulizer added to the amount of BDP on the impactor. Expressing the mass balance in terms of fractions, the fraction of BDP remaining in the nebulizer plus the fraction of BDP on the impactor should equal unity. As can be deduced from the fractions given in Table II, this was only the case with formulation II. In other cases, there was a net loss of BDP. In particular, for formulation III, only 80% of BDP was accounted for, and in formulation IV, the percent accounted for dropped to about 60%.

It is evident when the fraction of BDP collected on the impactor stage is plotted as a function of the cut-off diameter of the stage that suspensions of raw drug substance have a distribution of particles with a larger size and its distribution is more polydisperse. The nanoparticles have particles size distributions with 80% of the particles being less than 2.5 μm.

In Table III, the results from the fluorescein study are given. In comparing the mass exited, both formulations gave similar results of about 0.75. There was also no significant difference between the fractions of BDP and fluorescein remaining in the nebulizer. For the suspension, the fraction of BDP and fluorescein remaining were 88 and 89%, respectively. For the nanoparticles, the percents were 81 and 85 which are not statistically different from each other. In addition, there was no statistical difference in the fractions of BDP and fluorescein remaining in the nebulizer between formulations I and II. However, the fractions of BDP and fluorescein remaining are significantly greater than the fraction of total mass remaining for the suspension and nanoparticle formulations.

The fractions of BDP reaching the impactor were different between the two formulations. For the suspension, the fraction of fluorescein collected on the impactor was almost twice as high as the fraction of BDP. For the nanoparticles, the fraction of fluorescein was similar to that found with suspensions. The fraction of BDP collected on the impactor was much higher than observed with suspensions, but slightly less than that observed with fluorescein.

The final study was an examination of the particles after being subjected to the process of nebulization. Scanning electron microscopy was conducted of the nanoparticles deposited on the sixth stage of the impactor for the 2.5 and 0.1% nanoparticles

TABLE I

Formulation Components

Formulation	Form	[Surfactant]	Volume (mL)
I	Suspension	2.5%	1.85
II	Nanoparticle Dispersion	2.5%	1.85
III	Nanoparticle Dispersion	0.1%	1.85
IV	Nanoparticle Dispersion	0.1%	5.85

Formulation "I" is a comparative formulation not using nanoparticles.

TABLE II

Comparison of Nebulization Output Parameters as a Function of Formulate Effect of Nebulization Process on Resulting Aerosol Production. Results are expressed as the mean ± standard deviation, n = 3.

Formulation	Mass output rate (mg/sec)	Mass fraction remain.	BDP fraction remain.	BDP fraction on impactor
I	2.73 ± 0.5	0.69 ± 0.036	0.89 ± 0.013	0.082 ± 0.012
II	2.61 ± 0.14	0.51 ± 0.15	0.768 ± 0.23	0.184 ± 0.47
III	4.99 ± 0.31	0.67 ± 0.006	0.618 ± 0.025	0.174 ± 0.019
IV	4.35 ± 0.65	0.27 ± 0.015	0.289 ± 0.039	0.345 ± 0.15

TABLE III

Comparison of the nebulization of nanoparticle dispersions and suspensions of BDP containing a solution of fluorescein. Results are expressed as the mean ± deviation, n = 3.

Formulation	Mass fraction remaining	BDP fraction remaining	Fluorescein fraction remaining	BDP fraction on impactor	Fluorescein fraction on impactor
Suspension	0.76 ± 0.06	0.88 ± 0.046	0.89 ± 0.13	0.067 ± 0.02	0.122 ± 0.033
Nanoparticles	0.74 ± 0.017	0.81 ± 0.088	0.85 ± 0.065	0.11 ± 0.016	0.143 ± 0.020

We claim:

1. An aerosol of an aqueous dispersion of nanoparticles, wherein the nanoparticles comprise 0.1 to 60% (w/w) of insoluble beclomethasone particles having (1) an average particle size of less than about 400 nm, and (2) 0.1 to 90% (w/w) of a surface modifier adsorbed on the surface thereof.

2. An aerosol according to claim 1; wherein said surface modifier is selected from the group consisting of polyvinyl alcohol, polyvinylpyrrolidone tyloxapol, a polyoxamer, a polyoxamine dextran lecithin a dialkylester of sodium sulfosuccinic acid, sodium lauryl sulfate, an alkyl aryl polyether sulfonate, a polyoxyethylene sorbitan fatty acid ester, polyethylene glycol, a mixture of sucrose stearate and sucrose distearate, $C_{18}H_{37}CH_2(CON(CH_3)CH_2(CHOH)_4(CH_2OH)_2$, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxy propylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, and isononylphenoxypoly(glycidol).

3. A method for forming an aerosol of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble beclomethasone particles comprising:

a) providing an aqueous suspension of nanoparticles, wherein the nanoparticles comprise 0.1 to 60% (w/w) of insoluble beclomethasone particles having (1) an average particle size of less than about 400 nm, and (2) 0.1 to 90% (w/w) of a surface modifier adsorbed on the surface thereof; and

b) nebulizing said suspension so as to form an aerosol.

4. A method of treating a respiratory related illness of a mammal comprising:

administering an effective amount of an aerosol comprising an aqueous dispersion of nanoparticles, wherein said nanoparticles comprise 0.1 to 60% (w/w) of insoluble beclomethasone particles having (1) an average particle size of less than about 400 nm, and (2) 0.1 to 90% of a surface modifier adsorbed on the surface thereof, wherein droplets of the aerosol deposit in the respiratory tract of the mammal.

5. A method according to claim 4, wherein droplets of the aerosol deposit in the lung of the mammal.

6. The aerosol according to claim 1 or 2, wherein the beclomethasone particles are beclomethasone dipropionate particles.

7. The method according to any one of claims 3, 4, or 5, wherein the beclomethasone particles are beclomethasone dipropionate particles.

8. The method according to claim 3, wherein the surface modifier is selected from the group consisting of polyvinyl alcohol, polyvinylpyrrolidone, tyloxapol, a polyoxamer, a polyoxamine, dextran, lecithin, a dialkylester of sodium sulfosuccinic acid, sodium lauryl sulfate, an alkyl aryl polyether sulfonate, a polyoxyethylene sorbitan fatty acid ester, polyethylene glycol, a mixture of sucrose stearate and sucrose distearate, $C_{18}H_{37}CH_2(CON(CH_3)CH_2(CHOH)_4-(CH_2OH)_2$, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxy propylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, and isononylphenoxypoly(glycidol).

9. The method according to claim 4, wherein the surface modifier is selected from the group consisting of polyvinyl alcohol, polyvinylpyrrolidone, tyloxapol, a polyoxamer, a polyoxamine, dextran, lecithin, a dialkylester of sodium sulfosuccinic acid, sodium lauryl sulfate, an alkyl aryl polyether sulfonate, a polyoxyethylene sorbitan fatty acid ester, polyethylene glycol, a mixture of sucrose stearate and sucrose distearate, $C_{18}H_{37}CH_2(CON(CH_3)CH_2(CHOH)_4-(CH_2OH)_2$, carboxymethylcellulose calcium, carboxymethylcellul hydroxyethylcellulose, hydroxy propylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, and isononylphenoxypoly(glycidol).

10. The method according to claim 4, wherein the respiratory related illness is selected from the group consisting of seasonal rhinitis, perennial rhinitis, seasonal allergic (vasomotor) rhinitis, seasonal nonallergic (vasomotor) rhinitis, perennial allergic (vasomotor) rhinitis, and perennial nonallergic (vasomotor) rhinitis.

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APPENDIX B: EVIDENCE

2.
U.S. Patent No. 6,241,969
to Saidi et al.

US006241969B1

(12) **United States Patent**
Saidi et al.

(10) **Patent No.: US 6,241,969 B1**
(45) **Date of Patent: Jun. 5, 2001**

(54) **AQUEOUS COMPOSITIONS CONTAINING CORTICOSTEROIDS FOR NASAL AND PULMONARY DELIVERY**

(75) Inventors: **Zahir Saidi**, Philadelphia, PA (US); **Boris Klyashchitsky**, Newark, DE (US)

(73) Assignee: **Elan Corporation plc**, Dublin (IL)

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(58) **Field of Search** 424/450, 45, 198.1; 514/179, 180

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Primary Examiner—T. Moezie
(74) *Attorney, Agent, or Firm*—Synnestvedt & Lechner LLP

(57) **ABSTRACT**

The present invention provides compositions containing corticosteroid compounds as active agents for the treatment of ailments and diseases of the respiratory tract, particularly the lungs, by way of nasal and pulmonary administration. The corticosteroid compounds are present in a dissolved state in the compositions. The compositions can be formulated in a concentrated, essentially non-aqueous form for storage or in a diluted, aqueous-based form for ready delivery. In a preferred embodiment, the corticosteroid composition contains an ethoxylated derivative of vitamin E and/or a polyethylene glycol fatty acid ester as the high-HLB surfactant present in the formulation. The compositions are ideally suited for inhaled delivery with a nebulizer or for nasal delivery.

29 Claims, No Drawings

AQUEOUS COMPOSITIONS CONTAINING CORTICOSTEROIDS FOR NASAL AND PULMONARY DELIVERY

FIELD OF THE INVENTION

The present invention relates to pulmonary drug delivery compositions useful for the inhaled administration of corticosteroid compounds and the method of their administration. The delivery compositions are useful for the treatment of ailments and diseases of the lungs. Similar corticosteroid compositions may be used for nasal delivery.

BACKGROUND OF THE INVENTION

Delivery of therapeutic compounds directly to affected lung tissues has several advantages. The drug reaches the target tissue without first entering the systemic circulation and being subjected to dilution by the blood, binding to blood components, or metabolism by the liver and excretion by the kidneys. A high local concentration of drug can be achieved in the lungs while the systemic concentration is kept below that likely to cause adverse side effects. In addition, the apical side of the lung tissue—the side exposed directly to inspired air—can be treated with compounds that might not readily cross the endothelium or epithelium, which form barriers between the apical surface and the blood plasma. Similar considerations apply to the tissues lining the nasal passages and sinus cavities.

Several means have been developed to deliver compounds directly to the passages of the lung or nose. The most common form, especially for water-insoluble drugs, is a powder suspension that is propelled into the mouth while the patient inhales.

Propulsion is accomplished by use of pressurized gas or by any of a variety of mechanical means of entraining a fine powder into a gas or air stream. Common devices for this purpose include metered dose inhalers (MDIs), turbo inhalers, and dry powder inhalers. Each of these uses a different means of propulsion; however, a common characteristic is that once the therapeutic drug leaves the device it is, or becomes, a fine powder. In an MDI, the drug may be suspended or solubilized in a non-aqueous propellant, which is typically a chlorofluorocarbon or fluorinated hydrocarbon that is a liquid under pressure at room temperature. In turbo inhalers and dry powder inhalers, the drug is present in the form of a micronized powder.

The particle size distribution of the aerosolized drug compositions is very important to the therapeutic efficacy of the drug when delivered by inhalation. Studies of inhaled aerosols indicate that particles or droplets of greater than about 5 micrometers in mean aerodynamic diameter are effectively excluded from entry into the lungs and are captured in the nasal passages or throat and swallowed instead. Thus, the drug compounds delivered by these devices must be formulated in such a way that the mass median aerodynamic diameter (MMAD) is below 5 micrometers. In addition, even smaller particle sizes, on the order of 0.5 to 2.5 micrometers, are needed if the drug is to reach the alveolar sacs deep in the lungs. However, particles with aerodynamic diameter less than about 0.5 micrometers are likely to be exhaled before the drug is totally deposited on the lung surface.

Additional considerations for the use of powder-type drug delivery devices for inhalation include the limited amount of drug that can be contained in one or two puffs from the device and the need for the user to skillfully coordinate hand activation of the device with inhalation. This latter limitation

is particularly important for those patients who are disabled, children, or elderly.

Nebulizers offer an alternative method of administering therapeutic agents to the lungs. These devices work by means of an air jet or an ultrasonic pulse that is applied to a solution producing a fine mist. Therapeutic agents dissolved or suspended in the solution can be incorporated into the mist. The patient then breathes the mist in and out over the course of several minutes of treatment, during which 1 to 3 mL of the drug formulation is typically nebulized. Considerations of particle size mentioned above also apply to the droplet size of the mists. However, it is possible to rebreathe a portion of the mist during several minutes of treatment and increase the capture of the fine droplet fraction that can penetrate the lung most deeply. In addition, there is no need for coordination between hand action and breathing, making the nebulizer easier to use for patients. It may be possible, in some cases, to administer drugs not soluble in aqueous solution by nebulizing them in suspension. However, the droplet size of nebulized drug-containing suspensions cannot be smaller than that of the suspended particles. Therefore, the finer droplets produced from these systems would not contain any drug.

Thus, one limitation of nebulized formulations is that they are most suitable for those drug compounds that are sufficiently water soluble such that a therapeutic dose of the drug can be dissolved in from 1 to about 3 mL of aqueous solution. One way around this limitation is to formulate with polar organic solvents or aqueous solutions thereof. However, few organic solvents can be safely inhaled for prolonged periods. Most organic solvents that are currently approved for use in inhalation devices are propellants, such as chlorofluorocarbons (CFCs), which will soon be eliminated from manufacturing for environmental reasons, or the newer hydrofluorocarbons and low boiling hydrocarbons, all of which are expected to evaporate prior to penetrating the lungs. Such solvents can evaporate rapidly during nebulization and leave the drug behind in the device or in large particles that would be likely to be deposited in the mouth or throat rather than be carried to the lungs. Indeed, MDIs were developed to circumvent such problems.

Another way to overcome the solubility problem of the drug is to blend cosolvents such as ethanol, propylene glycol, or polyethylene glycol with water. However, there are limits to acceptable levels of these cosolvents in inhaled products. Typically, the cosolvents make up less than about 35% by weight of the nebulized composition, although it is the total dose of cosolvent as well as its concentration that determines these limits. The limits are set by the propensity of these solvents either to cause local irritation of lung tissue, to form hyperosmotic solutions which would draw fluid into the lungs, and/or to intoxicate the patient. In addition, most potential hydrophobic therapeutic agents are not sufficiently soluble in these cosolvent mixtures.

Thus, there is a need to develop improved systems that can solubilize water-insoluble drugs for nebulization, and to minimize the levels of cosolvent necessary to accomplish this. The ideal system would have a cosolvent concentration below about 15% and in certain cases below about 5%. It would consist of non-toxic ingredients and be stable for long periods of storage at room temperature. When nebulized, it would produce droplets having an MMAD less than about 5 micrometers.

Droplet size considerations are not as critical for sinus or nasal administration, but it is still important to use safe, non-irritating ingredients. An additional consideration for

both nasal and inhaled delivery is that some of the formulation will inevitably be tasted and swallowed. Therefore, acceptable taste and odor must be considered important parameters, especially for nebulized formulations where exposure is prolonged and where pediatric subjects form an important fraction of the probable patient population.

Anti-inflammatory corticosteroids, which are essentially water-insoluble drugs that act on inflammatory cells in the respiratory mucosa, are a type of therapeutic compounds in need of improved inhaled delivery. These steroids are useful in treating a variety of inflammatory diseases including asthma.

Asthma is a chronic obstructive disease of the lower airways. The major clinical and pathological features of asthma are (partially) reversible airflow limitations due to bronchial constriction, bronchial hyperreactivity to noxious stimuli such as allergens or cold air, and inflammation of the airways. Anti-inflammatory corticosteroids are useful in treating this last condition. They are the most effective group of therapeutic agents currently available for treating allergic asthma. The steroids suppress many inflammatory processes including inhibition of eosinophilia, epithelial shedding, and edema. The cellular basis of these actions is under active investigation.

Like other steroid hormone analogs, corticosteroids bind with high affinity to cytoplasmic receptor proteins in target cells. The receptor-steroid complexes migrate to the cell nucleus, where they interact with nuclear chromatin to control gene expression. The receptor binding is saturable and very small amounts of steroid suffice to elicit maximum cellular responses, including suppression of inflammation.

Anti-inflammatory steroids can act systemically as well as locally. Therefore, while systemic administration of anti-inflammatory steroids will diminish airway inflammation in asthmatics, it can also cause such adverse effects as general immunosuppression and imbalances in mineral metabolism. The corticosteroids commonly used in asthma treatment have a high ratio of topical to systemic potency. That is, these corticosteroids are highly active when delivered directly to the site of inflammation but relatively inactive when passed through the systemic circulation. The portion of an inhaled dose which is swallowed and absorbed through the intestine or absorbed through the lung tissue into the circulation is subjected to metabolism by the liver and converted to less active compounds with short half-lives. These metabolites are quickly eliminated from the blood, reducing the incidence of systemic side effects.

Among the most commonly used steroids are aldosterone, beclomethasone, betamethasone, budesonide, cloprednol, cortisone, cortivazol, deoxycortone, desonide, desoximetasone, dexamethasone, difluorocortolone, fluclorolone, flumethasone, flunisolide, fluocinolone, fluocinonide, fluocortin butyl, fluorocortisone, fluorocortolone, fluorometholone, flurandrenolone, fluticasone, halcinonide, hydrocortisone, icomethasone, meprednisone, methylprednisolone, mometasone, paramethasone, prednisolone, prednisone, tixocortol, triamcinolone, and others, and their respective pharmaceutically acceptable derivatives, such as beclomethasone diproprionate, dexamethasone 21-isonicotinate, fluticasone propionate, icomethasone enbutate, tixocortol 21-pivalate, triamcinolone acetonide, and others. Fortunately, some of these synthetic steroids have low potentials for systemic absorption because of their unique structures and metabolism.

Corticosteroids have usually been formulated as suspensions of micronized drug powder in chlorofluorocarbon vehicles or with chlorofluorocarbon-free propellants and delivered by metered dose inhaler. The choice of this type of carrier and apparatus was dictated by the fact that corticosteroids are very difficult to stabilize in aqueous media and frequently produce systems that exhibit crystal growth, precipitation, and/or aggregation of suspended or solubilized drug.

Corticosteroids have been formulated in different drug delivery systems for administration to the respiratory tract. U.S. Pat. No. 5,292,499 relates to reverse micelle colloidal dispersions of hydrophilic pharmaceutically active compounds prepared with aerosol CFC propellant formulations useful for topical, endopulmonary, nasal, or inhalation administration.

U.S. Pat. No. 5,208,226 describes the concept of using a novel combination therapy, which has greater efficacy and duration of bronchodilator action than previously known combinations and that permits the establishment of a twice daily dosing regimen. The effective treatment consists of administration of a stimulant bronchodilator, salmeterol, and/or a physiologically acceptable salt thereof, combined with beclomethasone dipropionate in a form suitable for inhalation such as a metered dose inhaler with dry powder or chlorofluorocarbon-containing formulations.

U.S. Pat. No. 5,474,759 discloses aerosol formulations that are substantially free of chlorofluorocarbons, and having particular utility in medicinal applications. The formulations contain a propellant (such as 1,1,1,2,3,3,3-heptafluoropropane), a medium-chain fatty acid propylene glycol diester, a medium-chain triglyceride, optionally a surfactant, and optionally auxiliary agents such as antioxidants, preservatives, buffers, sweeteners and taste masking agents. These formulations are used as carriers for the delivery of inhaled drugs such as albuterol, momestrasone, isoprenaline, disodium cromoglycate, pentamidine, ipratropium bromide, and salts and clathrates thereof.

Recently, several corticosteroid liposomal formulations have been under development. U.S. Pat. No. 5,192,528 discloses the delivery of corticosteroids by inhalation for treating a variety of lung diseases. The carrier consists of an aqueous suspension of sized liposomes containing the drug. This liposome-entrapped drug form is then aerosolized, using a pneumatic nebulizer, to deliver the drug to the lung. Cholesterol and/or cholesterol sulfate can be incorporated into the system to delay the release of corticosteroid from the liposomes in the lung environment. These formulations have many advantages over microcrystalline formulations, including utilization of otherwise water-insoluble materials, sustained pulmonary release, and facilitated intracellular delivery. However, some general problems pertaining to liposomes regarding manufacturing processes, the use of synthetic phopsholipids (such as dilauroylphosphatidylcholine), and the distribution patterns of aerosolized liposomes in the lung may cause difficulties in the wide application of this type of aerosolized formulation.

There are as yet no marketed, commercial liposomal, micellar, or microemulsion formulations available for pulmonary delivery of corticosteroids.

SUMMARY OF THE INVENTION

The present invention provides compositions suitable for administering a therapeutic dose of a corticosteroid to the respiratory tract and methods for the administration of said compositions.

In one embodiment, the corticosteroid composition contains from about 0.1 to about 20 percent by weight of a high-HLB surfactant component (HLB greater than about 10), for example, ethoxylated derivatives of Vitamin E such as tocopheryl polyethylene glycol 1000 succinate ("TPGS"). The HLB, or hydrophilic-lipophilic balance, is a measure on an arbitrary scale of the polarity of a surfactant or mixture of surfactants. For example, TPGS has an HLB between about 15 and 19. Generally, the corticosteroid composition contains the corticosteroid in an amount from about 5 μg/ml to about 1 mg/ml. The composition is aqueous-based, containing, at least about 70 weight percent of an aqueous phase that can include buffering, tonicity, taste-masking, and preservation additives.

The corticosteroid composition can also contain one or more pharmaceutically acceptable cosolvents to aid in the processing of the composition and to increase the solubility of the corticosteroid. Such cosolvents include mono- and polyvalent alcohols, such as propylene glycol, ethanol, and polyethylene glycol. Optionally, the corticosteroid compositions also can contain such components as low-HLB surfactants (HLB below about 8) and/or oils. Low-HLB surfactants include phospholipids, medium-chain mono- and diglycerides, and mixtures thereof. Useful pharmaceutically acceptable oils include triglycerides and propylene glycol diesters of medium-chain fatty acids.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compositions containing corticosteroid compounds as active agents for the treatment of ailments and diseases of the respiratory tract, particularly the lungs, by way of nasal and pulmonary administration. The compositions can be formulated such that they contain the corticosteroid active agent(s) in a dissolved state. The formulations can be stored either in a concentrated form to be diluted at the time of use or a ready-for-use, diluted state. The present invention also sets forth methods for using the compositions for nasal or inhaled delivery.

The corticosteroid compositions of the present invention are preferably formulated with ethoxylated derivatives of vitamin E as the high-HLB surfactant component. An example of a preferred high-HLB surfactant from this class of surfactants is tocopheryl polyethylene glycol 1000 succinate ("TPGS"). TPGS is commercially available from Eastman Chemical Company as "Vitamin E TPGS", and has been used as a water-soluble Vitamin E supplement for oral ingestion. It is a waxy solid at room temperature and has melting point around 40° C. It has been found that the use of TPGS in corticosteroid compositions is particularly advantageous due to the ability of TPGS to solubilize corticosteroids and to form a stable micellar solution upon dilution in an aqueous phase, and also due to the neutral taste of TPGS when used in a corticosteroid composition that is administered either nasally or by inhalation. Consequently, an embodiment of the present invention that is particularly well suited for ease of manufacturing is one in which the corticosteroid compound is initially dissolved in TPGS to form a "concentrate" that is diluted with an aqueous phase to form the final corticosteroid composition. This composition is a micellar solution because the concentration of TPGS is far above the critical micellar concentration (CMC) of TPGS, which is about 0.02 wt. percent in water at 37° C. This embodiment is easy to manufacture, has a low level of excipients, and has a neutral taste for inhalation delivery.

Compositions designed for inhaled administration have a level of the high-HLB surfactant in the final, diluted corticosteroid composition from about 0.1 to about 20, preferably from about 0.25 to about 15, and more preferably from about 0.5 to about 5, percent by weight. Compositions designed for nasal administration have a level of the high-HLB surfactant in the final, diluted corticosteroid composition from about 1 to about 20, preferably from about 2.5 to about 15 and more preferably from about 5 to about 10, percent by weight.

The corticosteroids that are useful in the present invention generally include any steroid produced by the adrenocortex, including glucocorticoids and mineralocorticoids, and synthetic analogs and derivatives of naturally occurring corticosteroids having anti-inflammatory activity. Examples of corticosteroids that can be used in the compositions of the invention include aldosterone, beclomethasone, betamethasone, budesonide, cloprednol, cortisone, cortivazol, deoxycortone, desonide, desoximetasone, dexamethasone, difluorocortolone, fluclorolone, flumethasone, flunisolide, fluocinolone, fluocinonide, fluocortin butyl, fluorocortisone, fluorocortolone, fluorometholone, flurandrenolone, fluticasone, halcinonide, hydrocortisone, icomethasone, meprednisone, methylprednisolone, paramethasone, prednisolone, prednisone, tixocortol, triamcinolone, and their respective pharmaceutically acceptable derivatives, such as beclomethasone diproprionate, dexamethasone 21-isonicotinate, fluticasone propionate, icomethasone enbutate, tixocortol 21-pivalate, and triamcinolone acetonide. Particularly preferred are compounds such as beclomethasone diproprionate, budesonide, flunisolide, fluticasone propionate, mometasone and triamcinolone acetonide.

The corticosteroid compound is present in the final, diluted corticosteroid composition designed for inhalation in an amount from about 5 μl/ml to about 5 mg/ml, preferably from about 10 μg/ml to about 1 mg/ml, and more preferably from about 20 μg/ml to about 500 μg/ml. For example, the preferred drug concentration is between about 20 and 100 μg/ml for beclomethasone dipropionate, between about 30 and 150 μg/ml for triamcinolone acetonide, and between about 50 and 200 μg/ml for budesonide, depending on the volume to be administered. By following the preferred methods of the present invention, relatively high solubilities of the corticosteroid can be achieved in an aqueous-based composition. The solubility of the corticosteroid can be greater than about 50, preferably greater than about 75, and more preferably greater than about 100, in some cases greater than about 150 or about 200, μg/ml.

Similarly, the corticosteroid compound is present in the final, diluted corticosteroid composition designed for nasal administration in an amount from about 50 μg/ml to about 10 mg/ml, preferably from about 100 μg/ml to about 2 mg/ml, and more preferably from about 300 μg/ml to about 1 mg/ml. For example, the preferred drug concentration is between about 200 and 900 μg/ml for beclomethasone dipropionate, between about 250 μg/ml and 1 mg/ml for triamcinolone acetonide, and between about 400 μg/ml and 1.6 mg/ml for budesonide, depending on the volume to be administered.

The corticosteroid composition can also contain various excipients that improve the storage stability of the composition, but which do not significantly affect the overall efficacy of the composition in its freshly prepared state. Such excipients include buffers, osmotic (tonicity-adjusting) agents, low toxicity antifoaming agents, and preservatives.

Buffers are used in the present compositions to adjust the pH to a range of between about 4 and about 8, preferably between about 4.5 to about 7, and more preferably between about 5 and about 6.8. The buffer species may be any

pharmaceutically approved buffer providing the aforementioned pH ranges, such as citrate, phosphate, malate, etc.

The osmotic agent can be used in the compositions to enhance the overall comfort to the patient upon delivery of the corticosteroid composition. It is preferred to adjust the osmolality of the composition to about 280–300 mOsm/kg. Such agents include any low molecular weight water-soluble species pharmaceutically approved for pulmonary and nasal delivery such as sodium chloride and glucose.

Preservatives can be used to inhibit microbial growth in the compositions. The amount of preservative is generally that which is necessary to prevent microbial growth in the composition for a storage period of at least six months. Examples of pharmaceutically acceptable preservatives include the parabens, benzalkonium chloride, thimerosal, chlorobutanol, phenylethyl alcohol, benzyl alcohol, and potassium sorbate.

Corticosteroid compositions that contain the high-HLB surfactant can be prepared as follows. TPGS will be used as the representative high-HLB surfactant for illustrative purposes. First, the TPGS may be heated to a temperature of at least about 40° C., preferably at least about 45° C., and generally about 45–60° C. The appropriate quantity of the corticosteroid compound is then dissolved in the molten TPGS at the same temperature, thus forming the concentrated corticosteroid composition. To achieve the final, diluted corticosteroid composition, the molten concentrated corticosteroid composition is slowly added under continuous stirring to an aqueous phase. The aqueous phase is preferably water containing the additives necessary to adjust the pH and tonicity, and preservatives if the formulation is intended for multiple use. It is preferred that the aqueous phase be heated prior to the addition of the molten corticosteroid concentrate to aid in dispersion. Generally, the aqueous phase should be heated to about 55–85° C., more preferably from about 60–70° C.

It is preferred that the diluted corticosteroid composition be formulated by first dissolving the drug in the molten TPGS and then dispersing this concentrate in the aqueous phase. If the drug is added to a prediluted mixture of TPGS and aqueous phase, it may not be possible to achieve the final desired concentration of the drug in a dissolved state. To ensure that the drug is solubilized and stable in the diluted composition, it is preferred that the level of the drug in the concentrated composition be from about 1 to about 30 mg/ml, preferably from about 2 to about 20 mg/ml, and more 5 preferably from about 2 to about 10 mg/ml prior to dilution. The level of water in the concentrated corticosteroid composition should be below 5% by weight, preferably below 2% by weight, and more preferably below 1% by weight, and in general, it is advantageous not to add any water to the concentrated corticosteroid composition.

The aqueous phase, which is composed of water and optionally buffering, tonicity, and/or preservation additives, is present in the diluted corticosteroid compositions containing TPGS in an amount of at least about 70, preferably at least about 80, more preferably at least 90, and even more preferably at least about 95, percent by weight. The various other additives, such as buffers, tonicity adjusting agents, and preservatives, are preferably blended into the compositions as part of the aqueous phase, and the use of the term "aqueous phase" is intended to include such components, if used.

It has been found that the inclusion of any one of a group of cosolvents in these TPGS corticosteroid compositions can aid in the processing of the compositions and in the solubilizing of the drug. Preferred cosolvents include mono- and polyvalent alcohols, such as propylene glycol, ethanol, glycerol, glycofurol (available as Tetraglycol from Sigma), ethoxydiglycol (available as Transcutol from Gattefosse), and polyethylene glycol (PEG) having an average molecular weight between about 200 and 4000, preferably between 200 and 1000, more preferably PEG 400, and combinations thereof. The cosolvents can be present individually in the final, diluted corticosteroid compositions in concentrations from about 0.1 to about 20, preferably from about 0.25 to about 15, more preferably from about 0.5 to about 5, and even more preferably from about 0.5 to about 2.5, percent by weight. The total level of cosolvents combined in the final, diluted corticosteroid compositions is from about 0.1 to about 20, preferably from about 0.25 to about 15, more preferably from about 0.5 to about 10, and even more preferably from about 0.5 to about 5, percent by weight.

When preparing the corticosteroid compositions, the cosolvents can be added to the molten TPGS, to the TPGS/drug concentrate, or to the aqueous phase in which the TPGS/drug concentrate will be dispersed. Any way, stable diluted corticosteroid compositions can be produced with the drug in a dissolved state. If the cosolvents are blended with the molten TPGS prior to the addition of the drug, the temperature of this concentrate can then be reduced during the dissolution process. In general, the temperature of the TPGS/cosolvent mixture can be maintained below about 50° C., preferably below about 45° C., in order to dissolve the drug. In some cases, such as when a volatile cosolvent like ethanol is used, no heating is necessary to achieve dissolution. In addition, when the concentrated composition contains a cosolvent, it is not necessary to heat the aqueous phase used as the dilution medium to form the diluted corticosteroid composition.

Alternatively, the drug can be first dissolved in the cosolvent or blend of cosolvents at 20–50° C. and then that solution is blended with the molten TPGS to form the concentrated corticosteroid composition.

Other preferred high-HLB surfactants that can be used in place of, or in admixture with, ethoxylated derivatives of vitamin E are polyethylene glycol fatty acid esters. The fatty acid moiety preferably has from about 8 to about 18 carbon atoms. A preferred polyethylene glycol fatty acid high-HLB surfactant product is "Solutol HS-15," available from BASF Fine Chemicals. Solutol HS-15 is a mixture of polyethyleneglycol 660 12-hydroxystearate (70%) and polyethylene glycol (30%). It is a white paste at room temperature that becomes liquid at about 30° C. and has an HLB of about 15. Aqueous solutions of this surfactant, like those of TPGS, have a neutral taste. Similar preferred manufacturing processes and behavior regarding the dissolution of drugs, dilution methods, and the addition of cosolvents apply to Solutol HS-15 as those mentioned above for TPGS.

The corticosteroid compositions can contain other high-HLB surfactants, such as ethoxylated hydrogenated castor oil (Cremophor RH40 and RH60, available from BASF), tyloxapol, sorbitan esters such as the Tween series (from ICI Surfactants) or the Montanox series (from Seppic), etc. The corticosteroid compositions preferably contain either, or both, of the ethoxylated derivatives of vitamin E or the polyethylene glycol fatty acid esters as all or part of the high-HLB surfactant component, and in general the sum of these two types of surfactants will account for at least 50%, preferably at least 75%, and more preferably at least 90% by wt. of the high-HLB surfactant component.

Optionally, low HLB surfactants, having an HLB value below about 8, can also be used in the present invention.

Examples of such low HLB surfactants include phospholipids, such as phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositol; and medium-chain mono- and diglycerides, i.e., mono- and di-glycerides of C_8 to C_{12} fatty acids, and mixtures thereof. The low HLB surfactants can be used in general at levels from about 0.1 to about 3 percent by weight in the diluted composition.

Optionally, an oil can also be incorporated into the compositions. Examples of pharmaceutically acceptable oil compounds include triglycerides and propylene glycol diesters of C_8 to C_{12} fatty acids such as the Captex series available from Abitec. Oils can be used in general in levels from about 1 to about 30 percent by weight in the concentrated compositions and from about 0.1 to about 3 percent by weight in the diluted composition.

It is necessary to add the drug to the compositions containing high-HLB and low HLB surfactants, and/or cosolvents, and/or the oil compounds, to form the concentrated corticosteroid compostion prior to dilution with the aqueous phase.

The diluted corticosteroid compositions using high-HLB surfactants such as TPGS or Solutol HS-15 to solubilize the drug are believed to be micellar compositions. This belief is based on the fact that the critical micelle concentration for both TPGS and Solutol HS-15 is about 0.02% by weight at 37° C., which is below their concentration in the diluted corticosteroid compositions. If an oil component is present with or without a low HLB surfactant, an oil-in-water (o/w) microemulsion may be formed as the diluted corticosteroid composition.

The aforementioned diluted compositions can be administered to the body in the form of an aerosol. For administration to the respiratory tract, particularly the lungs, a nebulizer is used to produce appropriately sized droplets. Typically, the particle size of the droplet produced by a nebulizer for inhalation is in the range between about 0.5 to about 5 microns. If it is desired that the droplets reach the lower regions of the respiratory tract, i.e., the alveoli and terminal bronchi, the preferred particle size range is between about 0.5 and about 2.5 microns. If it is desired that the droplets reach the upper respiratory tract, the preferred particle size range is between 2.5 microns and 5 microns. The nebulizer operates by directing pressurized air to fluidize the droplets of the diluted corticosteroid composition, which resultant aerosol is directed through a nozzle and subsequently through a baffle system that removes larger particles.

For the treatment of bronchial constriction, the diluted corticosteroid composition is prepared as described above. The corticosteroid for such treatment is preferably either beclomethasone dipropionate, betamethasone, budesonide, dexamethasone, flunisolide, fluticasone propionate, or triamcinolone acetonide, and is formulated in the concentrations set forth above. The daily dose of the corticosteroid is generally about 0.4 to 2 mg, depending on the drug and the disease, in accordance with the Physician's Desk Reference.

EXAMPLES

Various embodiments of the present invention are illustrated by the following examples, which should not be intended to limit the scope of the invention. The compositions of Examples 1, 2, 3, and 5 are suitable for inhalation via nebulization and the composition of Example 4 is suitable for nasal administration.

Example 1

The glucocorticoid beclomethasone dipropionate monohydrate was dissolved in premelted (50° C.) TPGS at concentrations of 2.8 and 6.3 mg per gram. These concentrates were kept at 50° C. during the entire solubilization process, which was about 15 min. While in this molten form, the concentrates were diluted at various volume ratios from 1:10 to 1:100 in various aqueous solutions such as hot (80° C.) deionized water, saline, malate buffer, citrate buffer, phosphate buffer, and 5% solutions of propylene glycol, PEG 200, or PEG 400 in any of the above. These diluted compositions were blended until any gel that may have formed when the TPGS concentrate came into contact with the aqueous phase was completely dispersed. Transparent, physically stable, diluted corticosteroid compositions without any precipitates were obtained containing about 28 to 420 μg/ml beclomethasone dipropionate. The diluted corticosteroid compositions were sterilized by passing them through a 0.22 micron sterile filter.

Example 2

Beclomethasone dipropionate monohydrate (4.2 mg) was dissolved in 995.8 mg of a binary liquid mixture of TPGS and ethanol (1:1 weight ratio) by briefly mixing at room temperature to form a concentrated corticosteroid composition. The concentrate was diluted 1:100 by volume in solutions of 5 wt.% PEG 400 in either deionized water, saline, or 20 mM malate, citrate, or phosphate buffer, by mixing for several minutes at room temperature. The resulting optically transparent, diluted corticosteroid compositions contained about 42 μg beclomethasone dipropionate per ml. The diluted corticosteroid compositions were sterilized by passing them through a 0.22 micron sterile filter.

The same concentrated corticosteroid composition was also diluted 1:50 by volume in the above-mentioned aqueous phases, and resulted in final formulations containing about 84 μg beclomethasone dipropionate per ml. These diluted formulations were physically and chemically stable for over a year at 5° C., 25° C./60% RH and 40° C./75% RH.

Example 3

Several corticosteroids—beclomethasone dipropionate, budesonide, and triamcinolone acetonide—were dissolved in binary mixtures of TPGS and a cosolvent selected from the group of ethanol, propylene glycol, PEG 200 and PEG 400. The weight ratio of TPGS to cosolvent was 1:1, and the resulting drug concentrations were between 1.4 and 4.0 mg/gram. It was necessary to heat the TPGS/propylene glycol and the TPGS/PEG mixtures to approximately 45° C. for several minutes in order to dissolve the drugs, but dissolution could be achieved in the TPGS/ethanol mixture at room temperature. The concentrates were diluted 1:50 by volume in an aqueous phase (5% wt. PEG 400 in deionized water) resulting in clear solutions containing from 28 μg to 80 μg per mL. The diluted corticosteroid compositions were sterilized by passing them through a 0.22 micron sterile filter.

Example 4

The composition of this example is suitable for nasal administration. Beclomethasone dipropionate monohydrate (2.8 mg) was dissolved in 997.2 mg of a 2:1 w/w mixture of PEG 200 and TPGS and then diluted (1:6.65 by volume) with deionized water. The final transparent solution contained 420 μg of beclomethasone dipropionate per mL of solution. The composition of the formulation is given below. The tonicity can be adjusted to about 300 mOsm/kg by the addition of glucose or sodium chloride.

Component	Weight Percent Concentrate Mixture	Wt/Vol. Percent After 1:6.65 Dilution
TPGS	33.24	5
PBG 200	66.48	10
Beclomethasone dipropionate	0.28	0.042
Deionized water	—	q.s.

The diluted corticosteroid compositions were sterilized by passing them through a 0.22 micron sterile filter.

Example 5

In order to assess the stability profiles of some of the corticosteroid compositions described in this invention, four formulations were made with the weight compositions given in the following table.

Component	Form. 1	Form. 2	Form. 3	Form. 4
Beclomethasone dipropionate	42 μg/g	42 μg/g	42 μg/g	42 μμg/g
TPGS	1%	1%	0.5%	0.5%
Polyethylene glycol 400	—	1%	5%	5%
Ethyl Alcohol (190 Proof)	—	—	0.5%	0.5%
Deionized Water	q.s.	q.s.	q.s	—
0.9% NaCl Solution	—	—	—	q.s.

Formulations were stored in glass vials and blow-molded polyethylene ampules for the duration of the study. Various tests were used to assess the physical and chemical stability of the corticosteroid compositions given above.

Size and distribution of the dispersed material droplets in the aqueous solution of the above compositions were determined using a quasi-elastic light scattering technique. The experimental equipment consisted of a BI-200SM Goniometer and BI9000AT Digital Correlator from Brookhaven Instrument Corporation, and a Thorn EMI Electron tube for detection powered by a high voltage power supply, delivering 2000 volts, from Bertan Associates. A helium-neon laser from Spectra Physics was the light source, with a wavelength of 632.8 nm. The droplet size of the dispersed phase in all formulations before nebulization was about 10 nm, and remained constant for the duration of the study.

The MMAD and the corresponding geometric standard deviation (GSD) of the nebulized corticosteroid compositions were determined at time zero of the study. Saline was used as a reference. The experiments were done using a system consisting of a Proneb compressor and a Pari LC Plus Reusable Nebulizer (Pari Respiratory Equipment, Inc., Richmond, Va.) equipped with an adapted mouthpiece, connected in series with an Andersen cascade impactor (Andersen Airsampler Inc., Atlanta, Ga.). A vacuum pump was connected to the outlet of the cascade impactor, and between them was an air flow controller which indicated a flow of about 28.3 L/min. A cascade impactor is a mechanical model of human lung, containing seven stages and a filter before the outlet, which represent increasing depths of penetration. The amount of excipients deposited on each plate was determined by the increase in the plate dry weight. Analogous results were obtained when determining the MMAD from the drug mass on each plate. This showed that the drug travels in same manner as the excipients.

Formulation	MMAD (μm)	% GSD
1	2.939	2.81
2	2.294	2.46
3	2.795	2.48
4	2.165	2.42
Saline	2.216	2.16

Analysis for the corticosteroid content and degradation products in the above compositions was performed by HPLC. A Shimadzu LC 10A was used with a Supelcosil LC-318 column and UV/VIS detector monitoring absorbance at a wavelength of 254 nm. The isocratic method used 60% acetonitrile in deionized water at a flow rate of about 1.5 mL/min for 15 min. Visual examinations of the corticosteroid compositions under crossed polarized light films and by the naked eye were made on a weekly basis. These examinations were done in order to observe over time whether there was any phase separation, drug precipitate, turbidity or change in color. Results of the stability study at 40° C./75% RH after 12 weeks are shown below. From these data it can be concluded that the tested formulations are physically stable, meaning that there was no phase separation or precipitation of the drug under stressed conditions. No degradation of the corticosteroid was observed. Similar results were obtained from samples which were stored at 5° C. and 25° C.

Formulation	Drug content, t = 0, μg/mL	Drug content, t = 12 wk μg/mL glass vials	LDPE ampules
1	43.09	45.08 (104.6)	45.49 (105.6)
2	42.55	43.80 (102.9)	44.32 (104.2)
3	41.96	42.91 (102.3)	43.06 (102.6)
4	41.31	41.55 (100.6)	41.26 (99.9)

What is claimed is:

1. An aerosolized composition for administering a therapeutic dose of a corticosteroid to respiratory tract, consisting essentially of:

(a) from 5 μg/mL to about 5 mg/mL of a dissolved corticosteroid;

(b) from about 0.1 to about 20 percent by weight of a pharmaceutically acceptable, high-HLB surfactant component containing one or more surfactants having an HLB of greater than 10, wherein The high-HLB surfactant component comprises at least 50% by weight of an ethoxylated derivative of vitamin E; and

(c) at least about 70 weight percent aqueous phase.

2. The composition of claim **1** wherein the corticosteroid comprises beclomethasone dipropionate.

3. The composition of claim **1** wherein the corticosteroid comprises budesonide.

4. The composition of claim **1** wherein the corticosteroid comprises triamcinolone acetonide.

5. The composition of claim **1** wherein the corticosteroid comprises fluticasone propionate.

6. The composition of claim **1** wherein the corticosteroid comprises flunisolide.

7. The composition of claim **1** wherein the high-HLB surfactant component comprises at least 50% by weight tocopheryl polyethylene glycol 1000 succinate.

8. The composition of claim 1 wherein the ethoxylated derivative of vitamin E comprises at least 75% by weight of the high-HLB surfactant component.

9. The composition of claim 1 wherein the ethoxylated derivative of vitamin E comprises at least 90% by weight of the high-HLB surfactant component.

10. The composition of claim 1 wherein the high-HLB surfactant component comprises at least 75% by weight tocopheryl polyethylene glycol 1000 succinate.

11. The composition of claim 1 wherein the high-HLB surfactant component comprises at least 90% by weight tocopheryl polyethylene glycol 1000 succinate.

12. An aerosolized composition for administering a therapeutic dose of a corticosteroid to respiratory tract, composing:

(a) from 5 μg/mL to about 5 mg/mL of a dissolve corticosteroid;

(b) from about 0.1 to about 20 percent by weight of a pharmaceutically acceptable, high-HLB surfactant component containing one or more surfactants having an HLB of greater than 10, wherein the high-HLB surfactant component comprises at least 50% by weight of an ethoxylated derivative of vitamin E; and

(c) at least about 70 weight percent aqueous phase.

13. The composition of claim 12 wherein the high-HLB surfactant component comprises at least 75 percent by weight of an ethoxylated derivative of vitamin E.

14. The composition of claim 12 wherein the high-HLB surfactant component comprises at least 90 percent by weight of an ethoxylated derivative of vitamin E.

15. The composition of claim 12 further comprising from about 0.1 to about 20 percent by weight of a pharmaceutically acceptable cosolvent comprising propylene glycol, polyethylene glycol having a molecular weight between about 200 and 4000, glycerol, ethoxydiglycol, glycofurol, and ethanol, or a combination thereof.

16. The composition of claim 12 further comprising from about 0.1 to about 3 percent by weight of a low HLB surfactant having an HLB below about 8.

17. The composition of claim 12 further comprising from about 0.1 to about 3 percent by weight of an oil.

18. The composition of claim 12 wherein the high-HLB surfactant component comprises at least 75% by weight tocopheryl polyethylene glycol 1000 succinate.

19. The composition of claim 12 wherein the high-HLB surfactant component comprises at least 90% by weight tocopheryl polyethylene glycol 1000 succinate.

20. A method for administering a therapeutic dosage of an aerosolized corticosteroid to respiratory tract of a patient in need thereof, comprising:

(a) providing a corticosteroid composition comprising:

(1) from 5 μg/mL to about 5 mg/mL of a dissolved corticosteroid;

(2) from about 0.1 to about 20 percent by weight of a pharmaceutically acceptable, high-HLB surfactant component containing one or more surfactants having an HLB of greater than 10, wherein the high-HLB surfactant component comprises at least 50% by weight of an ethoxylated derivative of vitamin E; and

(3) at least about 70 weight percent aqueous phase;

(b) aerosolizing the corticosteroid composition; and

(c) administering a therapeutically effective dosage of the aerosolized composition to said patient by inhalation.

21. The method of claim 20 wherein the corticosteroid composition consists essentially of said corticosteroid, said aqueous phase, and said high-HLB surfactant.

22. The method of claim 20 wherein the ethoxylated derivative of vitamin E comprises at least 75% by weight of the high-HLB surfactant component.

23. The method of claim 20 wherein the high-HLB surfactant component comprises at least 75% by weight tocopheryl polyethylene glycol 1000 succinate.

24. A method for administering a therapeutic dosage of an aerosolized corticosteroid composition to the nasal passage of a patient in need thereof, comprising:

(a) providing a corticosteroid composition comprising:

(1) from 5 μg/mL to about 5 mg/L of a dissolved corticosteroid;

(2) from about 0.1 to about 20 percent by weight of a pharmaceutically acceptable, high-HLB surfactant component containing one or more surfactants, having an HLB of greater than 10, wherein high-HLB surfactant component comprises at least 50% by weight of an ethoxylated derivative of vitamin E; and

(3) at least about 70 weight percent aqueous phase,

(b) administering a therapeutically effective dosage of the corticosteroid composition by nasal inhalation to said patient.

25. The method of claim 24 wherein the ethoxylated derivative of vitamin E comprises at least 75% by weight of the high-HLB surfactant component.

26. The method of claim 24 wherein the high-HLB surfactant component comprises at least 75% by weight tocopheryl polyethylene glycol 1000 succinate.

27. A method of preparing a diluted corticosteroid composition containing a dissolved corticosteroid, composing:

(a) dissolving a corticosteroid compound into a molten pharmaceutically acceptable high-HLB surfactant component comprising one or more surfactants having an HLB greater than 10, and wherein the high-HLB surfactant component comprises at least 50 percent by weight of an etioxylated derivative of vitamin E;

(b) subsequently blending The molten high-HLB surfactant component containing the dissolved corticosteroid with an aqueous phase,

wherein the aqueous phase is present in an amount of at least about 70 weight percent, and the high-HLB surfactant component is present in an amount of from about 0.1 to about 20 weight percent, of the diluted corticosteroid composition.

28. The method of claim 27 wherein the ethoxylated derivative of vitamin E comprises at least 75% by weight of the high-HLB surfactant component.

29. The method of claim 27 wherein the high-HLB surfactant component comprises at least 75% by weight tocopheryl polyethylene glycol 1000 succinate.

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APPENDIX B: EVIDENCE

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(71) Applicant: NANOSYSTEMS L.L.C. [US/US]; Building 1, 1250 South Collegeville Road, Collegeville, PA 19426 (US).

(72) Inventors: WOOD, Ray, W.; 421 Toland Drive, Fort Washington, PA 19034 (US). DECASTRO, Lan; 1186 Queen Lane, West Chester, PA 19382 (US). BOSCH, H., William; Bryn Mawr, PA 19010 (US).

(74) Agents: WEST, Paul, B.; Ladas & Parry, 26 West 61st Street, New York, NY 10023 (US) et al.

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(57) Abstract

There is disclosed an aerosol comprising droplets of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble therapeutic or diagnostic agent particles having a surface modifier on the surface thereof. There is also disclosed a method for making the aerosol and methods for treatment and diagnosis using the aerosol.

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AEROSOLS CONTAINING NANOPARTICLE DISPERSIONS

FIELD OF THE INVENTION

The present invention is directed to the field of nanoparticles and particularly in an aerosol form.

BACKGROUND OF THE INVENTION

Delivery of therapeutic agent to the respiratory tract is important for both local and systemic treatment of disease. With the conventional techniques, delivery of agents to the lung is extremely inefficient. Attempts to develop respirable aqueous suspensions of poorly soluble compounds have been unsuccessful. Micronized therapeutic agents suspended in aqueous media are too large to be delivered by aerosolized aqueous droplets. With conventional processes, it is estimated that only about 10 to 20% of the agent reaches the lung. Specifically, there is loss to the device used to deliver the agent, loss to the mouth and throat and with exhalation. These losses lead to variability in therapeutic agent levels and poor therapeutic control. In addition, deposition of the agent to the mouth and throat can lead to systemic absorption and undesirable side effects.

The efficiency of respiratory drug delivery is largely determined by the particle size distribution. Large particles (greater than 10 m) are primarily deposited on the back of the throat. Greater than 60% of the particles with sizes between 1 and 10 m pass with the air stream into the upper bronchial region of the lung where most are deposited. With particles less than about 1 μm, essentially all of the particles enter the lungs and pass into the peripheral alveolar region; however, about 70% are exhaled and therefore are lost.

In addition to deposition, the relative rate of absorption and rate of clearance of the therapeutic agent must be considered for determining the amount of therapeutic agent that reaches the site of action. Since 99.99% of the available area is located in the peripheral alveoli, rapid absorption can be realized with delivery of the particles to the periphery. For clearance, there is also differences between the central and peripheral regions of the lung. The peripheral alveolar region does-not have ciliated cells but relies on macrophage engulfment for

particle clearance. This much slower process can significantly extend the time during which the particles reside in the lung thereby enhancing the therapeutic or diagnostic effect. In contrast, particles deposited in the upper respiratory tract are rapidly cleared by mucociliary escalator. That is, the particles are trapped in the mucous blanket coating the lung surface and are transported to the throat. Hence, this material is either swallowed or removed by coughing.

While it has long been known that smaller droplets of an aerosol reach deeper into the respiratory system (Current Concepts in the Pharmaceutical Sciences: Dosage and Bioavailability, J. Swarbrick Ed., Lea and Febiger, Philadelphia, PA, 1973, pp. 97-148) these have largely been of theoretical interest. Simply knowing that smaller droplets of aersol can be delivered deeper into the respiratory system does not solve the problem of incorporating sufficient therapeutic agent into the aerosol to be efficient, particularly where the therapeutic agent is only slightly soluble in the liquid for the aerosol.

Nanoparticles, described in U.S. Patent No. 5,145,684, are particles consisting of a poorly soluble therapeutic or diagnostic agent onto which are adsorbed a non-crosslinked surface modifier, and which have an average particle size of less than about 400 nanometers (nm). However, no mention is made of attempts to nebulize (aerosolize or atomize are equivalent terms for the purpose of this disclosure) these compositions and it is not apparent that nebulizing these composition would provide useful aerosols or that there would be any advantage for doing so.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided an aerosol comprising droplets of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble therapeutic or diagnostic agent particles having a surface modifier on the surface thereof.

In another aspect of the invention, there is provided a method for forming an aerosol of a nanoparticle dispersion, said nanoparticles comprising insoluble therapeutic or diagnostic agent particles having a surface modifier on the surface thereof, said method comprising the steps of:

a) providing a suspension of said nanoparticles;

b) nebulizing said suspension so as to form an aerosol.

In yet another aspect of the invention, there is provided a method of treating a mammal comprising the steps of:

a) forming an aerosol of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble therapeutic agent particles having a surface modifier on the surface thereof;

b) administering said aerosol to the respiratory system of said mammal.

In yet another embodiment, there is provided a method of diagnosing a mammal, said method comprising

a) forming an aerosol of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble diagnostic imaging agent particles having a surface modifier on the surface thereof;

b) administering said aerosol to the respiratory system of said mammal; and

c) imaging said imaging agent in said respiratory system.

DETAILED DESCRIPTION OF THE INVENTION

The compositions of the invention are aerosols. Aerosols can be defined for the present purpose as colloidal systems consisting of-very finely divided liquid droplets dispersed in and surounded by a gas. The droplets in the aerosols typically have a size less than about 50 microns in diameter although droplets of a much smaller size are possible.

The aerosols of the present invention are particularly useful in the treatment of respiratory related illnesses such as asthma, emphysema, respiratory distress syndrome, chronic bronchitus, cystic fibrosis and acquired immune deficiency syndrome including AIDS related pneumonia.

The aerosols of the invention are made by nebulizing the nanoparticle containing solution using a variety of known nebulizing techniques. Perhaps the simplest of systems is the "wo-phase" system which consists of a solution or a suspension of active ingredient, in the present case, a nanoparticle containing a therapeutic or diagnostic agent, in a liquid propellent. Both liquid and vapor phases are present in a pressurized container and when a valve on the container is opened, liquid propellent containing the nanoparticle dispersion is released. Depending on the nature of the ingredients and the nature of the valve mechanism, a fine aersol mist or aersol wet spray is produced.

There are a variety of nebulisers that are available to produce the aerosols of the invention including small volume nebulizers. Compressor driven nebulizers incorporate jet technology and use compressed air to generate the

aersol. Commercially available devices are available from Healthdyne Technologies Inc; Invacare Inc.; Mountain Medical Equipment Inc.; Pari Respiratory Inc.; Mada Mediacal Inc.; Puritan-Bennet; Schuco Inc.; Omron Healthcare Inc.; DeVilbiss Health Care Inc; and Hospitak Inc.

Ultrasonic nebulizers deliver high medication output and are used by patients-suffering from severe asthma, or other severe respiratory related illnesses.

The particles comprise a therapeutic or diagnostic agent. (therapeutic agents are sometimes referred to as drugs or pharmaceuticals. The diagnostic agent referred to is typically a contrast agent such as an x-ray contrast agent but can also be other diagnostic materials.) The therapeutic or diagnostic agent exists as a discrete, crystalline phase. The crystalline phase differs from a non-crystalline or amorphous phase which results from precipitation techniques, such as described in EPO 275,796.

The invention can be practiced with a wide variety of therapeutic or diagnostic agents. The therapeutic or diagnostic agent preferably is present in an essentially pure form. The therapeutic or diagnostic agent must be poorly soluble and dispersible in at least one liquid medium. By "poorly soluble" it is meant that the therapeutic or diagnostic agent has a solubility in the liquid dispersion medium of less than about 10 mg/mL, and preferably of less than about 1 mg/mL. A preferred liquid dispersion medium is water. However, the invention can be practiced with other liquid media in which a therapeutic or diagnostic agent is poorly soluble and dispersible including, for example, aqueous salt solutions, safflower oil and solvents such as ethanol, t-butanol, hexane and glycol. The pH of the aqueous dispersion media can be adjusted by techniques known in the art.

Suitable therapeutic or diagnostic agents can be selected from a variety of known classes of therapeutic or diagnostic agents including, for example, analgesics, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives (hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, corticosteroids, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (antiparkinsonian

agents), haemostatics, immuriological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators and xanthines. Preferred therapeutic or diagnostic agents include those intended for oral administration and intravenous administration. A description of these classes of therapeutic agents and diagnostic agents and a listing of species within each class can be found in Martindale, *The Extra Pharmacopoeia,* Twenty-ninth Edition, The Pharmaceutical Press, London, 1989. The therapeutic or diagnostic agents are commercially available and/or can be prepared by techniques known in the art.

Preferred diagnostic agents include the x-ray imaging agent WIN-8883 (ethyl 3,5-diacetamido-2,4,6triiodobenzoate) also known as the ethyl ester of diatrazoic acid (EEDA), WIN 67722, i.e., (6-ethoxy-6-oxohexyl-3,5-bis(acetamido)- 2,4,6-triiodobenzoate; ethyl-2-(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy)butyrate (WIN 16318); ethyl diatrizoxyacetate (WIN 12901); ethyl 2-(3,5bis(acetamido)-2,4,6-triiodobenzoyloxy)propionate (WIN 16923); N-ethyl 2-(3,5-bis(acetamido)-2,4,6triiodobenzoyloxy acetamide (WIN 65312); isopropyl 2(3,5-bis(acetamido)-2,4,6-triiodobenzoyloxy) acetamide (WIN 12855); diethyl 2-(3,5-bis(acetamido)-2,4,6triiodobenzoyloxy malonate (WIN 67721); ethyl 2-(3,5bis(acetamido)-2,4,6-triiodobenzoyloxy) phenylacetate (WIN 67585); propanedioic acid, [[3,5-bis(acetylamino)2,4,5-triodobenzoyl]oxy]-,bis(1-methyl)ester (WIN 68165); and benzoic acid, 3,5-bis(acetylamino)-2,4,6triodo-, 4-(ethyl-3-ethoxy-2-butenoate) ester (WIN 68209). Suitable diagnostic agents are also disclosed in U.S. Patent No. 5,260,478; U.S. Patent No. 5,264,610; U.S. Patent No. 5,322,679 and U.S. Patent No. 5,300,739.

Preferred contrast agents include those which are expected to disintegrate relatively rapidly under physiological conditions, thus minimizing any particleassociated inflammatory response. Disintegration may result from enzymatic hydrolysis, solubilization of carboxylic acids at physiological pH, or other mechanisms. Thus, poorly soluble iodinated carboxylic acids such as iodipamide, diatrizoic acid, and metrizoic acid, along with hydrolytically labile iodinated species such as WIN 67721, WIN 12901, WIN 68165, and WIN 68209 or others may be preferred.

Surface Modifiers

Suitable surface modifiers can preferably be selected from known organic and inorganic pharmaceutical excipients. Such excipients include various polymers, low molecular weight oligomers, natural products and surfactants. Preferred surface modifiers include nonionic and ionic surfactants.

Representative examples of surface modifiers include gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens™, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxy propylcellulose, hydroxypropylmethylcellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidone (PVP). Most of these surface modifiers are known pharmaceutical excipients and are described in detail in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986.

Particularly preferred surface modifiers include polyvinylpyrrolidone, tyloxapol, poloxamers such as Pluronics™ F68 and F108, which are block copolymers of ethylene oxide and propylene oxide, and polyxamines such as Tetronics™ 908 (also known as Poloxamine™ 908), which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine, available from BASF, dextran, lecithin, dialkylesters of sodium sulfosuccinic acid, such as Aerosol OTs™, which is a dioctyl ester of sodium sulfosuccinic acid, available from American Cyanimid, Duponols™ P, which is a sodium lauryl sulfate, available from DuPont, Tritons™ X-200, which is an alkyl aryl polyether sulfonate, available from Rohn and Haas, Tween™ 20 and Tweens™ 80, which are polyoxyethylene sorbitan fatty acid esters, available from ICI Specialty Chemicals; Carbowaxs™ 3550 and 934, which are polyethylene glycols available from Union Carbide; Crodestas™ F-110, which is a mixture of sucrose stearate and sucrose distearate, available from Croda Inc., Crodestas™ SL-40, which is available from Croda, Inc., and SA9OHCO,

which is $C_{18}H_{37}CH_2(CON(CH_3)CH_2(CHOH)_4(CH_2OH)_2$. Surface modifiers which have been found to be particularly useful include Tetronics™ 908, the Tweenss™, Pluronics™ F-68 and polyvinylpyrrolidone. Other useful surface modifiers 15 include:

decanoyl-N-methylglucamide;
n-decyl β-D-glucopyranoside;
n-decyl β-D-maltopyranoside;
n-dodecyl β-D-glucopyranoside;
n-dodecyl β-D-maltoside;
heptanoyl-N-methylglucamide;
n-heptyl-β-D-glucopyranoside;
n-heptyl β-D-thioglucoside; n-hexyl β-D-glucopyranoside;
nonanoyl-N-methylglucamide;
n-noyl β-D-glucopyranoside;
octanoyl-N-methylglucamide;
n-octyl-β-D-glucopyranoside;
octyl β-D-thioglucopyranoside; and the like.

Another useful surface modifier is tyloxapol (a nonionic liquid polymer of the alkyl aryl polyether alcohol type; also known as superinone or triton). This surface modifier is commercially available and/or can be prepared by techniques known in the art.

Another preferred surface modifier is p-isononylphenoxypoly(glycidol) also known as Olin-lOG™ or Surfactant 10-G, is commercially available as lOG™ from Olin Chemicals, Stamford, Connecticut.

Non-Ionic Surface Modifiers

Preferred surface modifiers can be selected from known non-ionic surfactants, including the poloxamines such as Tetronic™ 908 (also known as Poloxamine™ 908), which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine, available from BASF, or Tetronic™ 1508 (T-1508), or a polymer of the alkyl aryl polyether alcohol type, such as tyloxapol.

The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two-or more surface modifiers can be used in combination.

Tyloxapol

Tyloxapol (4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde) is a preferred surface modifier and is a nonionic liquid polymer of the alkyl aryl polyether alcohol type. Tyloxapol, also known as "Superinone", is disclosed as useful as a nonionic surface active agent in a lung surfactant composition in U.S. Patent No. 4,826,821 and as a stabilizing agent for 2-dimethylaminoethyl 4-n-butylaminobenzoate in U.S. Patent No. 3,272,700.

Tyloxapol may be associated with the nanoparticles and may function as a surface modifier, as a stabilizer, and/or as a dispersant. Alternatively, the tyloxapol may serve other purposes. Tyloxapol may serve all three functions. The tyloxapol may serve as a stabilizer and/or a dispersant, whereas another compound acts as a surface modifier.

Auxiliary Surface Modifiers

Particularly preferred auxiliary surface modifiers are those which impart resistance to particle aggregation during sterilization and include dioctylsulfosuccinate (DOSS), polyethylene glycol, glycerol, sodium dodecyl sulfate, dodecyl trimethyl ammonium bromide and a charged phospholipid such as dimyristoyl phophatidyl glycerol. The surface modifiers are commercially available and/or can be prepared by techniques known in the art. Two or more surface modifiers can be used in combination.

Block Copolymer Surface Modifiers

One preferred surface modifier is a block copolymer linked to at least one anionic group. The polymers contain at least one, and preferably two, three, four or more anionic groups per molecule.

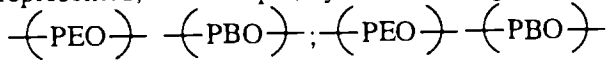
Preferred anionic groups include sulfate, sulfonate, phosphonate, phosphate and carboxylate groups. The anionic groups are covalently attached to the nonionic block copolymer. The nonionic sulfated polymeric surfactant has a molecular weight of 1,000-50,000, preferably 2,000-40,000 and more preferably 3,000- 30,000. In preferred embodiments, the polymer comprises at least about 50%, and more preferably, at least about 60% by weight of hydrophilic units, e.g., alkylene oxide units. The reason for this is that the presence of a major weight proportion of hydrophilic units confers aqueous solubility to the polymer.

A preferred class of block copolymers useful as surface modifiers herein includes sulfated block copolymers of ethylene oxide and propylene oxide.

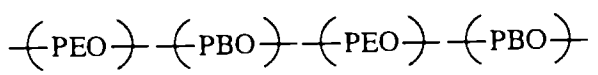
These block copolymers in an unsulfated form are commercially available as Pluronics™. Specific examples of the unsulfated block copolymers include F68, F108 and F127.

Another preferred class of block copolymers useful herein include tetrafunctional block copolymers derived from sequential addition of ethylene oxide and propylene oxide to ethylene diamine. These polymers, in an unsulfated form, are commercially available as Tetronics™ .

Another preferred class of surface modifiers contain at least one polyethylene oxide (PEO) block as the hydrophilic portion of the molecule and at least one polybutylene oxide (PBO) block as the hydrophobic portion. Particularly preferred surface modifiers of this class are diblock, triblock, and higher block copolymers of ethylene oxide and butylene oxide, such as are represented, for example, by the following structural formula:



; and



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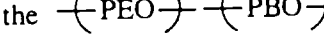
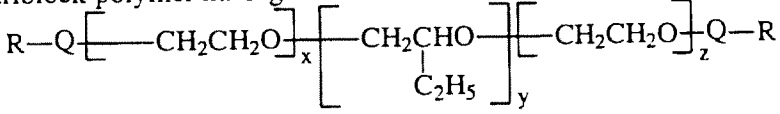
The block copolymers useful herein are known compounds and/or can be readily prepared by techniques well known in the art.

Highly preferred surface modifiers include triblock copolymers of the 

having molecular weights of 3800 and 5000 which are commercially available from Dow Chemical, Midland, Michigan, and are referred to as B20-3800 and B20-5000. These surface modifiers contain about 80% by weight PEO. In a preferred embodiment, the surface modifier is a triblock polymer having the structure:



Q is an anionic group

wherein R is H or a metal cation such as Na+, K+ and the like,
x is 15-700,
Y iS 5-200 and
z is 15-700. 30

Grinding

The described particles can be prepared in a method comprising the steps of dispersing a therapeutic or diagnostic agent in a liquid dispersion medium

and applying mechanical means in the presence of grinding media to reduce the particle size of the therapeutic or diagnostic agent to an effective average particle size of less than about 400 nm. The particles can be reduced in size in the presence of a surface modifier. Alternatively, the particles can be contacted with a surface modifier after attrition.

The therapeutic or diagnostic agent selected is obtained commercially and/or prepared by techniques known in the art in a conventional coarse form. It is preferred, but not essential, that the particle size of the coarse therapeutic or diagnostic agent selected be less than about 10 mm as determined by sieve analysis. If the coarse particle size of the therapeutic or diagnostic agent is greater than about 100 mm, then it is preferred that the particles of the therapeutic or diagnostic agent be reduced in size to less than 100 mm using a conventional milling method such as airjet or fragmentation milling.

The coarse therapeutic or diagnostic agent selected can then be added to a liquid medium in which it is essentially insoluble to form a premix. The concentration of the therapeutic or diagnostic agent in the liquid medium can vary from about 0.1 - 60%, and preferably is from 5 - 30% (w/w). It is preferred, but not essential, that the surface modifier be present in the premix. The concentration of the surface modifier can vary from about 0.1 to about 90%, and preferably is 1-75%, more preferably 20-60%, by weight based on the total combined weight of the therapeutic or diagnostic agent and surface modifier. The apparent viscosity of the premix suspension is preferably less than about 1000 centipoise.

The premix can be used directly by subjecting it to mechanical means to reduce the average particle size in the dispersion to less than 1000 nm. It is preferred that the premix be used directly when a ball mill is used for attrition. Alternatively, the therapeutic or diagnostic agent and, optionally, the surface modifier, can be dispersed in the liquid medium using suitable agitation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates-visible to the naked eye. It is preferred that the premix be subjected to such a premilling dispersion step when a recirculating media mill is used for attrition. Alternatively, the therapeutic or diagnostic agnet and, optionally, the surface modifier, can be dispersed in the iquid medium using suitable agitiation, e.g., a roller mill or a Cowles type mixer, until a homogeneous dispersion is observed in which there are no large agglomerates visible to the naked eye. It is preferred tha tthe premix be subjected

to such a premilling dispersion step when a recirculating media mill is used for attrition.

The mechanical means applied to reduce the particle size of the therapeutic or diagnostic agent conveniently can take the form of a dispersion mill. Suitable dispersion mills include a ball mill, an attritor mill, a vibratory mill, and media mills such as a sand mill and a bead mill. A media mill is preferred due to the relatively shorter milling time required to provide the intended result, desired reduction in particle size. For media milling, the apparent viscosity of the premix preferably is from about 100 to about 1000 centipoise. For ball milling, the apparent viscosity of the premix preferably is from about 1 up to about 100 centipoise. Such ranges tend to afford an optimal balance between efficient particle fragmentation and media erosion.

Preparation Conditions

The attrition time can vary widely and depends primarily upon the particular mechanical means and processing conditions selected. For ball mills, processing times of up to five days or longer may be required. On the other hand, processing times of less than 1 day (residence times of one minute up to several hours) have provided the desired results using a high shear media mill.

The particles must be reduced in size at a temperature which does not significantly degrade the therapeutic or diagnostic agent. Processing temperatures of less than about 30 - 40 C are ordinarily preferred. If desired, the processing equipment can be cooled with conventional cooling equipment. The method is conveniently carried out under conditions of ambient temperature and at processing pressures which are safe and effective for the milling process. For example, ambient processing pressures are typical of ball mills, attritor mills and vibratory mills. Control of the temperature, e.g., by jacketing or immersion of the milling chamber in ice water are contemplated. Processing pressures from about 1 psi (0.07 kg/cm2) up to about 50 psi (3.5 kg/cm2) are contemplated. Processing pressures from about 10 psi (0.7 kg/cm2) to about 20 psi 1.4 kg/cm2)

The surface modifier, if it was not present in the premix, must be added to the dispersion after attrition in an amount as described for the premix above. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonication step, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20 - 80 kHz for a time of about 1 to 120

seconds.

After attrition is completed, the grinding media is separated from the milled particulate product (in either a dry or liquid dispersion form) using conventional separation techniques, such as by filtration, sieving through a mesh screen, and the like.

Grinding Media

The grinding media for the particle size reduction step can be selected from rigid media preferably spherical or particulate in form having an average size less than about 3 mm and, more preferably, less than about 1 mm. Such media desirably can provide the particles with shorter processing times and impart less wear to the milling equipment. The selection of material for the grinding media is not believed to be critical. We have found that zirconium oxide, such as 95% ZrO2 stabilized with magnesia, zirconium silicate, and glass grinding media provide particles having levels of contamination which are believed to be acceptable for the preparation of pharmaceutical compositions. However, other media, such as stainless steel, titania, alumina, and 95% ZrO2 stabilized with yttrium, are expected to be useful. Preferred media have a density greater than about 3 g/cm3.

Polymeric Grinding Media

The grinding media can comprise particles, preferably substantially spherical in shape, e.g., beads, consisting essentially of polymeric resin. Alternatively, the grinding media can comprise particles comprising a core having a coating of the polymeric resin adhered thereon.

In general, polymeric resins suitable for use herein are chemically and physically inert, substantially free of metals, solvent and monomers, and of sufficient hardness and friability to enable them to avoid being chipped or crushed during grinding. Suitable polymeric resins include crosslinked polystyrenes, such as polystyrene crosslinked with divinylbenzene, styrene copolymers, polycarbonates, polyacetals, such as Delrin™, vinyl chloride polymers and copolymers, polyurethanes, polyamides, poly(tetrafluoroethylenes), e.g., Teflon™, and other fluoropolymers, high density polyethylenes, polypropylenes, cellulose ethers and esters such as cellulose acetate, polyhydroxymethacrylate, polyhydroxyethyl acrylate, silicone containing polymers such as polysiloxanes and the like. The polymer can be biodegradable. Exemplary biodegradable

polymers include poly(lactides), poly(glycolide) copolymers of lactides and glycolide, polyanhydrides, poly(hydroxyethyl methacylate), poly(imino carbonates), poly(N-acylhydroxyproline)esters, poly(N-palmitoyl hydroxyproline) esters, ethylene-vinyl acetate copolymers, poly(orthoesters), poly(caprolactones), and poly(phosphazenes). In the case of biodegradable polymers, contamination from the media itself advantageously can metabolize in vivo into biologically acceptable products which can be eliminated from the body.

The polymeric resin can have a density from 0.8 to 3.0 g/cm3. Higher density resins are preferred inasmuch as it is believed that these provide more efficient particle size reduction.

The media can range in size from about 0.1 to 3 mm. For fine grinding, the particles preferably are from 0.2 to 2 mm, more preferably, 0.25 to 1 mm in size.

In a particularly preferred method, a therapeutic or diagnostic agent is prepared in the form of submicron particles by grinding the agent in the presence of a grinding media having a mean particle size of less than about 75 microns.

The core material of the grinding media preferably can be selected from materials known to be useful as grinding media when fabricated as spheres or particles. Suitable core materials include zirconium oxides (such as 95% zirconium oxide stabilized with magnesia or yttrium), zirconium silicate, glass, stainless steel, titania, alumina, ferrite and the like. Preferred core materials have a density greater than about 2.5 g/cm3. The selection of high density core materials is believed to facilitate efficient particle size reduction.

Useful thicknesses of the polymer coating on the core are believed to range from about 1 to about 500 microns, although other thicknesses outside this range may be useful in some applications. The thickness of the polymer coating preferably is less than the diameter of the core.

The cores can be coated with the polymeric resin by techniques known in the art. Suitable techniques include spray coating, fluidized bed coating, and melt coating. Adhesion promoting or tie layers can optionally be provided to improve the adhesion between the core material and the resin coating. The adhesion of the polymer coating to the core material can be enhanced by treating the core material to adhesion promoting procedures, such as roughening of the core surface, corona discharge treatment, and the like.

Continuous Grinding

In a preferred grinding process, the particles are made continuously rather than in a batch mode. The continuous method comprises the steps of continuously introducing the therapeutic or diagnostic agent and rigid grinding media into a milling chamber, contacting the agent with the grinding media while in the chamber to reduce the particle size of the agent, continuously removing the agent and the grinding media from the milling chamber, and thereafter separating the agent from the grinding media.

The therapeutic or diagnostic agent and the grinding media are continuously removed from the milling chamber. Thereafter, the grinding media is separated from the milled particulate agent (in either a dry or liquid dispersion form) using conventional separation techniques, in a secondary process such as by simple filtration, sieving through a mesh filter or screen, and the like. Other separation techniques such as centrifugation may also be employed.

In a preferred embodiment, the agent and grinding media are recirculated through the milling chamber. Examples of suitable means to effect such recirculation include conventional pumps such as peristaltic pumps, diaphragm pumps, piston pumps, centrifugal pumps and other positive displacement pumps which do not use sufficiently close tolerances to damage the grinding media. Peristaltic pumps are generally preferred.

Another variation of the continuous process includes the use of mixed media sizes. For example, larger media may be employed in a conventional manner where such media is restricted to the milling chamber. Smaller grinding media may be continuously recirculated through the system and permitted to pass through the agitated bed of larger grinding media. In this embodiment, the smaller media is preferably between about 1 and 300 mm in mean particle size and the larger grinding media is between about 300 and 1000 mm in mean particle size.

Precipitation Method

Another method of forming the desired nanoparticle dispersion is by microprecipitation. This is a method of preparing stable dispersions of therapeutic and diagnostic agents in the presence of a surface modifying and colloid stability enhancing surface active agent free of trace of any toxic solvents or solubilized heavy metal inpurities by the following procedural steps:

1. Dissolving the therapeutic or diagnostic agent in aqueous

base with stirring,

2. Adding above #1 formulation with stirring to a surface active surfactant (or surface modifiers) solution to form a clear solution, and

3. Neutralizing above formulation #2 with stirring with an appropriate acid solution. The procedure can be followed by:

4. Removal of formed salt by dialysis or diafiltration and

5. Concentration of dispersion by conventional means.

This microprecipitation process produces dispersion of therapeutic or diagnostic agents with Z-average particle diameter less than 400 nm (as measured by photon correlation spectroscopy) that are stable in particle size upon keeping under room temperature or refrigerated conditions. Such dispersions also demonstrate limited particle size growth upon autoclave-decontamination conditions used for standard blood-pool pharmaceutical agents.

Step 3 can be carried out in semicontinuous, continuous batch, or continuous methods at constant flow rates of the reacting components in computercontrolled reactors or in tubular reactors where reaction pH can be kept constant using pH-stat systems. Advantages of such modifications are that they provide cheaper manufacturing procedures for large-scale production of nanoparticulate dispersion systems.

Additional surface modifier may be added to the dispersion after precipitation. Thereafter, the dispersion can be mixed, e.g., by shaking vigorously. Optionally, the dispersion can be subjected to a sonicationstep, e.g., using an ultrasonic power supply. For example, the dispersion can be subjected to ultrasonic energy having a frequency of 20-80 kHz for a time of about 1 to 120 seconds.

In a preferred embodiment, the above procedure is followed with step 4 which comprises removing the formed salts by diafiltration or dialysis. This is done in the case of dialysis by standard dialysis equipment and by diafiltration using standard diafiltration equipment known in the art. Preferably, the final step is concentration to a desired concentration of the agent dispersion. This is done either by diafiltration or evaporation using standard equipment known in this art.

An advantage of microprecipitation is that unlike milled dispersion, the final product is free of heavy metal contaminants arising from the milling media that must be removed due to their toxicity before product is formulated.

A further advantage of the microprecipitation method is that unlike

solvent precipitation, the final product is free of any trace of trace solvents that may be toxic and must be removed by expensive treatments prior to final product formulation.

In another preferred embodiment of the microprecipitation process, a crystal growth modifier is used. A crystal growth modifier is defined as a compound that in the co-precipitation process incorporates into the crystal structure of the microprecipitated crystals of the pharmaceutical agent, thereby hindering growth or enlargement of the microcrystalline precipitate, by the so called Ostwald ripening process. A crystal growth modifier (or a CGM) is a chemical that is at least 75% identical in chemical structure to the pharmaceuticl agent. By "identical" is meant that the structures are identical atom for atom and their connectivity. Structural identity is charactarized as having 75% of the chemical structure, on a molecular weight basis, identical to the therapeutic or diagnostic agent. The remaining 25% of the structure may be absent or replaced by different chemical structure in the CGM. The crystal growth modifier is dissolved in step #1 with the therapeutic or diagnostic agent.

Particle Size

As used herein, particle size refers to a number average particle size as measured by conventional particle size measuring techniques well known to those skilled in the art, such as sedimentation field flow fractionation, photon correlation spectroscopy, or disk centrifugation. When photon correlation spectroscopy (PCS) is used as the method of particle sizing the average particle diameter is the Z-average particle diameter known to those skilled in the art. By "an effective average particle size of less than about 1000 nm" it is meant that at least 90% of the particles have a weight average particle size of less than about 1000 nm when measured by the above-noted techniques. In preferred embodiments, the effective average particle size is less than about 400 nm and more preferrably less than about 300nm. In some embodiments, an effective average particle size of less than about 100 nm has been achieved. With reference to the effective average particle size, it is preferred that at least 95% and, more preferably, at least 99% of the particles have a particle size less than the effective average, e.g., 1000 nm. In particularly preferred embodiments essentially all of the particles have a size less than 1000 nm. In some embodiments, essentially all of the particles have a size less than 400 nm.

Ratios

The relative amount of therapeutic or diagnostic agent and surface modifier can vary widely and the optimal amount of the surface modifier can depend, for example, upon the particular therapeutic or diagnostic agent and surface modifier selected, the critical micelle concentration of the surface modifier if it forms micelles, the hydrophilic lipophilic balance (HLB) of the stabilizer, the melting point of the stabilizer, its water solubility, the surface tension of water solutions of the stabilizer, etc. The surface modifier preferably is present in an amount of about 0.1-10 mg per square meter surface area of the therapeutic or diagnostic agent. The surface modifier can be present in an amount of 0.1-90%, preferably 20-60% by weight based on the total weight of the dry particle.

Diagnosis

A method for diagnostic imaging for use in medical procedures in accordance with this invention comprises administering to the body of a test subject in need of a diagnostic image an effective contrast producing amount of the diagnostic image contrast composition. In addition to human patients, the test subject can include mammalian species such as rabbits, dogs, cats, monkeys, sheep, pigs, horses, bovine animals and the like. Thereafter, at least a portion of the body containing the administered contrast agent is exposed to x-rays or a magnetic field to produce an x-ray or magnetic resonance image pattern corresponding to the presence of the contrast agent. The image pattern can then be visualized.

Any x-ray visualization technique, preferably, a high contrast technique such as computed tomography, can be applied in a conventional manner. Alternatively, the image pattern can be observed directly on an x-ray sensitive phosphor screen-silver halide photographic film combination or by use of a storage phosphor screen.

Visualization with a magnetic resonance imaging system can be accomplished with commercially available magnetic imaging systems such as a General Electric 1.5 T Sigma imaging system [lH resonant frequency 63 . 9 megahertz (MHz)]. Commercially available magnetic resonance imaging systems are typically characterized by the magnetic field strength used, with a field strength of 2.0 Tesla as the current maximum and 0.2 Tesla as the current minimum. For a given field strength, each detected nucleus has a characteristic frequency. For example, at a field strength of 1.0 Tesla, the resonance frequency

for hydrogen is 42.57

10 MHz; for phosphorus-31 it is 17.24 MHz; and for sodium23 it is 11.26 Mhz.

A contrast effective amount of the diagnostic agent containing composition is that amount necessary to provide tissue visualization with, for example, magnetic resonance imaging or x-ray imaging. Means for determining a contrast effective amount in a particular subject will depend, as is well known in the art, on the nature of the magnetically reactive material used, the mass of the subject being imaged, the sensitivity of the magnetic resonance or x-ray imaging system and the like.

After administration of the compositions, the subject mammal is maintained for a time period sufficient for the administered compositions to be distributed throughout the subject and enter the tissues of the mammal. Typically, a sufficient time period is from about 20 minutes to about 90 minutes and, preferably from about 20 minutes to about 60 minutes. The following examples are presented for a further understanding of the invention.

Example 1 Using the Therapeutic Agent Beclomethasone

Materials. Beclomethasone diproprionate (BDP) and polyvinyl alcohol (PVA) were obtained from Sigma Chemical Co. (St. Louis, MO) and used as received. All other chemicals were analytical/reagent grade or better.

Nanoparticle Preparation and Characterization. Nanoparticles were prepared by media milling a suspension of 5% beclomethasone diproprionate in an aqueous solutions of PVA. Thus, the PVA was the surface modifier. The resulting particle size distribution was determined by dynamic light scattering. The particle size distribution was periodically monitored throughout the course of the study.

Nebulization. A gas cylinder of compressed air was used as the source, which was equipped with a pressure regulator. Oxygen connecting tubing joined from the regulator to the Puritan-Bennet Raindrop nebulizer (Lenexa, KA). One exit port of the T-connector of the nebulizer was blocked with a #2 rubber stopper. The other exit port was fitted with Tygon tubing (1/2" id). This in turn led initially to a calibrated flow meter from which the flow rate was set before each experiment. After calibration, the gas flow was stopped by shutting off the main cylinder valve. The flow meter was removed, and the nebulizer was connected to a Y-tube with 24/40 joints by tubing (1/2" id, 6" length). The Y-tube was connected to the cascade impactor (Andersen Mark I, Andersen

Samplers Ind. Atlanta, GA) by a constructed stainless steel adapter consisting of a tapered side that fit within the 24/40 ground glass joint and a cylindrical section with rubber o-ring gasket that fit into the top of the cascade impactor. The air flow rate through the impactor was drawn by a vacuum pump and regulated by a calibrated flow meter to the recommended 28.3 L/min.

Preliminary studies indicated that pressures between 20 and 40 psig had little effect on either the performance of the nebulizer or the resulting aerosol size distribution. Thus, the pressure was kept constant at 40 psig. Studies of the effect of flow rate on nebulizer performance and aerosol size distribution were also conducted. As the flow rate was decreased from 5 to 2 L/min, aerosol particles had progressively larger mean aerodynamic diameter. At a flow rate 8 L/min, there was excessive foaming. Thus, all studies were conducted at a flow rate of 6 L/min.

Suspension and Nanoparticle Nebulization. Formulations for nebulization consisted of a 0.2% beclomethasone diproprionate dispersions with PVA. The nebulizers contained either a volume of 2 mL or 6 mL. Two concentrations of PVA were used which were prepared by diluting the original 5% (w/v) nanoparticle dispersion with a PVA solution having the same PVA concentration as the original dispersion concentration or with water. The nebulizer was filled, and aliquots of the solution were taken for subsequent determination of drug concentration. The weight was also determined. The nebulization process was initiated by opening the valve on the main gas cylinder, and the length of time until foaming or sputtering of the nebulizer was determined, and additional aliquots were taken for analysis. The fraction of mass exiting the nebulizer was calculated from the weight difference of the nebulizer before and after nebulization. This was coupled with the time required for nebulization of the dispersion to yield the mass output rate in terms of the milliliters of dispersion nebulized/unit time and the nebulizer output in terms of the volume of dispersion nebulized/liter of air were determined.

Aliquots taken from the nebulizer were diluted with 50% (v/v) ethanol in water, and the absorbance determined at 240 nm. With measurement of the absorbance of appropriate standards, the concentration of BDP was calculated. From the masses of the nebulizer before and after nebulization and the BDP concentrations, the fraction of BDP remaining in the nebulizer was calculated. The mass of BDP collected on the cascade impactor and the aerosol particle size distribution was determined by extracting the impactor stages with 10 mL of the

ethanol/water solution. Aliquots were taken and the absorbances and subsequent concentration were determined. The mass median aerodynamic diameter and geometric standard deviation of the particle distribution was obtained by plotting the cumulative mass on the stages of the impactor as a function of the log of the cut-off diameter. With the cumulative mass determined from the cascade impactor and the initial amount of BDP placed in the nebulizer, the fraction of BDP reaching the impactor was calculated.

To assess the fractionation of the dispersion, the nanoparticles and suspensions were diluted with PVA solutions containing 0.1% sodium fluorescein. Nebulization was conducted as described above. Since fluorescein has significant absorbance at both 490 and 240 nm while BDP has absorbance only at 240 nm, the absorbance of the diluted aliquots was determined at these two wavelengths. The concentration of fluorescein was determined from the absorbance at 490 nm and the measured absorptivity. In determining the concentrations of BDP, the contribution from the absorbance of fluorescein at 240 nm was subtracted based on the absorbance determined at 490 and the correction for the differences in the absorptivity at these two wavelength.

Scanning Electron Microscopy. SEM was performed on nanoparticles after nebulization. Two dispersions were prepared containing 0.1 and 2.5% surfactant. These were placed in the nebulizer and 2 cm rectangular glass microscope slides were placed on every stage of the impactor. The glass slides were removed and sputtered 5 with platinum. Micrographs were obtained with a JEOL 840-II ElectroScan Environmental ESEM (Peabody, Mass.).

RESULTS

Nanoparticles of beclomethasone diproprionate in 2.5% polyvinyl alcohol had a particle size distribution of 0.26 ± 0.13 mm. This size remained constant throughout the course of the study; neither was there any evidence of chemical instability. In addition, particle size of the diluted dispersions remained constant for at least the duration of the experiment.

For nebulization, four formulations were tested. These are listed in Table I. The first was a suspension of raw drug substance BDP in 2.5% surfactant with a volume of 2 mL. The second was composed of a dispersion of nanoparticles thereby allowing direct comparison to the suspension formulation. The third was also a colloidal dispersin, but the surfactant concentration was smaller at 0.1%. The fourth was similar to the third but contained a larger volume

of 6 mL.

In Table II, the results from the nebulization of the four formulations were given. The second column provides the mass output rate which was the rate at which the total mass of the dispersion exists the nebulizer. Formulations I and II are similar as were formulations III and IV. The difference between these two sets of formulations is that I and II had a surfactant concentration of 2.5%, whereas III and IV had a surfactant concentration of 0.1%.

The third column reflects the total mass fraction of dispersion remaining in the nebulizer. The fraction of mass remaining was between 0.27 and 0.69 indicating considerable amount of material remained in the nebulizer. In addition, formulations I, II and III were similar, but formulation IV had a significantly lower mass fraction remaining in the nebulizer. Formulation IV is distinct from the others in that it contained an initial volume of 6 mL.

In the next column, the fraction of BDP remaining in the nebulizer is given. These fractions ranged from 0.29 to 0.89. In comparing the fractions remaining, formulation I, which contained the suspension, had about 90% of BDP remain in the nebulizer. In contrast, formulation III which contained 0.1% surfactant, had a significantly lower fraction of BDP remain in the nebulizer. An even more dramatic drop in fraction remaining was observed with formulation IV which had a low surfactant concentration as well as a larger volume.

It is also noteworthy to compare the fraction of BDP remaining relative to the fraction of total mass remaining in the nebulizer. With formulation I, there was a significantly greater fraction of BDP relative to the total mass remaining. Numerically this is also true for formulation II: however, there was more variability in these measurements which had no statistical difference in the fractions remaining. In formulations III and IV, there was no difference.

The fraction of BDP reaching the nebulizer is also given in Table II. It is seen that only about 7% of the BDP presented as a suspension or raw drug substance reaches the impactor. In comparison, the use of nanoparticles led to a significantly higher fraction reaching the impactor. These ranged from 0.17 to over 0.34. In formulations II and III which contained 2 mL of dispersion, about 18% of BDP reached the impactor. In the large volume formulation IV, almost 35% of BDP reached the impactor.

Finally, it is evident that the amount of BDP that was originally placed in the nebulizer should equal the amount of BDP remaining in the nebulizer added to the amount of BDP on the impactor. Expressing the mass

balance in terms of fractions, the fraction of BDP remaining in the nebulizer plus the fraction of BDP on the impactor should equal unity. As can be deduced from the fractions given in Table II, this was only the case with formulation II. In other cases, there was a net loss of BDP. In particular, for formulation III, only 80% of BDP was accounted for,and in formulation IV,the percent accounted for dropped to about 60%.

It is evident when the fraction of BDP collected on the impactor stage is plotted as a function of the cut-off diameter of the stage that suspensions of raw drug substance have a distribution of particles with a larger size and its distribution is more polydisperse. The nanoparticles have particles size distributions with 80% of the particles being less than 2.5 mm.

In Table III, the results from the fluorescein study are given. In comparing the mass exited, both formulations gave similar results of about 0.75. There was also no significant difference between the fractions of BDP and fluorescein remaining in the nebulizer. For the suspension, the fraction of BDP and fluorescein remaining were 88 and 89%, respectively. For the nanoparticles, the percents were 81 and 85 which are not statistically different from each other. In addition, there was no statistical difference in the fractions of BDP and fluorescein remaining in the nebulizer between formulations I and II. However, the fractions of BDP and fluorescein remaining are significantly greater than the fraction of total mass remaining for the suspension and nanoparticle formulations.

The fractions of BDP reaching the impactor were different between the two formulations. For the suspension, the fraction of fluorescein collected on the impactor was almost twice as high as the fraction of BDP. For the nanoparticles, the fraction of fluorescein was similar to that found with suspensions. The fraction of BDP collected on the impactor was much higher than observed with suspensions, but slightly less than that observed with fluorescein.

The final study was an examination of the particles after being subjected to the process of nebulization. Scanning electron microscopy was conducted of the nanoparticles deposited on the sixth stage of the impactor for the 2.5 and 0.1% nanoparticles.

Table I

Formulation Components

Formulation	Form	[Surfactant]	Volume (mL)
I	Suspension	2.5%	1.85
II	Nanoparticle Dispersion	2.5%	1.85
III	Nanoparticle Dispersion	0.1%	1.85
IV	Nanoparticle Dispersion	0.1%	5.85

Formulation "I" is a comparative formulation not using nanoparticles.

Table II

Comparison of Nebulization Output Parameters as a Function of Formulate Effect of Nebulization Process on Resulting Aerosol Production. Results are expressed as the mean + standard deviation, n=3.

Formulation	Mass Output Rate (mg/sec)	Mass Fraction Remain.	BDP Fraction Remain	BDP Fraction Remain
I	2.73 ± 0.5	0.69 ± 0.036	0.89 ± 0.013	0.082 ± 0.012
II	2.61 ± 0.14	0.51 ± 0.15	0.768 ± 0.23	0.184 ± 0.47
III	4.99 ± 0.31	0.67 ± 0.006	0.618 ± 0.025	0.174 ± 0.019
IV	4.35 ± 0.65	0.27 ± 0.015	0.289 ± 0.039	0.345 ± 0.15

Table III

Comparison of Nebulization of Nanoparticle Dispersions and Suspensions of BDP Containing a Solution of Fluorescein

Form-ulation	Mass Fraction Remain-ing	BDP Fraction Remaining	Fluorescein Fraction Remaining	BDP Fraction on Impactor	Fluorescein Fraction On Impactor
Suspen-sion	0.76 ± 0.06	0.88 ± 0.046	0.89 ± 0.13	0.067 ± 0.02	0.122 ± 0.033
Nano-particles	0.74 ± 0.17	0.81 ± 0.088	0.85 ± 0.065	0.11 ± 0.016	0.143 ± 0.020

Example 2 Using a Contrast Agent

In this example, a suspension of WIN 68209 (30%) in aqueous F108 surfactant (6%) was prepared by conventional roller milling techniques (jar mill, zirconium silicate beads, 7 days milling time). The mean particle size of the resultant distribution was 196 nm. The formulation was administered to an anesthetized rabbit as follows: Several mL of formulation was placed in an ultrasonic nebulizer (DeVilbiss AeroSonic (TM)) which was connected in-line with a mechanical ventilator, terminating in a suitable endotracheal tube. The rabbit was then intubated and administered the nebulized formulation for several minutes. Subsequent computed tomography (CT) scans of the rabbit's pulmonary region showed the presence of radiopaque contrast agent in the region.

The invention has been described with particular reference to preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

Claims:

1. An aerosol comprising droplets of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble therapeutic or diagnostic agent particles having a surface modifier on the surface thereof.

2. An aerosol according to claim 1 wherein said therapeutic agent is beclomethasone dipropionate.

3. An aerosol according to claim 1 wherein said diagnostic agent is benzoic acid, 3,5-bis(acetylamino)2,4,6-triodo-, 4-(ethyl-3-ethoxy-2-butenoate) ester (WIN 68209).

4. A method for forming an aerosol of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble therapeutic or diagnostic agent particles having a surface modifier on the surface thereof, said method comprising the steps of:

a) providing a suspension of said nanoparticles; and

b) nebulizing said suspension so as to form an aerosol.

5. A method of treating a mammal comprising the steps of:

a) forming an aerosol of an aqueous dispersion of nanoparticles, said nanoparticles comprising insoluble therapeutic agnet particles having a surface modifier oon the surface thereof;

b) administering said aerosol to the respiratory system of said mammal.

6. A method according to claim 5 wherein said aerosol is administered in a manner such that it reaches the lung.

7. A method according to claim 6 wherein said nanoparticles contain beclomethazone.

8.A method of diagnosing a mammal, said method comprising the steps of:

a) forming an aerosol of an aqueous dispersion of

nanoparticles, said nanoparticles comprising insoluble diagnostic imaging agent particles having a surface modifier on the surface thereof; and

b) administering said aerosol to the respiratory system of said mammal; and

c) imaging said imaging agent in said respiratory system.

9. A method according to claim 8 wherein said diagnostic imaging agent is benzoic acid, 3,5bis(acetylamino)-2,4,6-triodo-, 4-(ethyl-3-ethoxy-2butenoate) ester (WIN 68209).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02346

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 9/12
US CL :424/45, 46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/45, 46

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,145,684 (LIVERSIDGE ET AL.) 08 September 1992, see entire document.	1-9
Y	WO 92/08446 (GALAXO GROUP LTD.) 29 May 1992, see entire document.	1-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 08 APRIL 1996	Date of mailing of the international search report 30 APR 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RAJ BAWA, Ph.D. Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet)(July 1992)*

APPENDIX B: EVIDENCE

4.
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to Biggadike et al.

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(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2003/0073676 A1

Biggadike et al. (43) Pub. Date: Apr. 17, 2003

(54) FORMULATION CONTAINING ANTI-INFLAMMATORY ANDROSTANE DERIVATIVES

(76) Inventors: **Keith Biggadike**, Stevenage (GB); **Amyn P. Sayani**, Mississauga, CA (US); **Ian Buxton**, Mississauga, CA (US); **Kenton Reed**, Mississauga, CA (US)

Correspondence Address:
DAVID J LEVY, CORPORATE INTELLECTUAL PROPERTY
GLAXOSMITHKLINE
FIVE MOORE DR., PO BOX 13398
RESEARCH TRIANGLE PARK, NC 27709-3398 (US)

(21) Appl. No.: **10/066,951**

(22) Filed: **Feb. 4, 2002**

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/958,050, filed on Oct. 2, 2001, which is a continuation of application No. PCT/GB01/03495, filed on Aug. 3, 2001.

(30) **Foreign Application Priority Data**

Aug. 5, 2000 (GB) 0019172.6

Publication Classification

(51) **Int. Cl.**[7] **A61K 31/58**; A61K 31/57

(52) **U.S. Cl.** **514/179**; 514/176

(57) **ABSTRACT**

There is provided according to the invention a pharmaceutical formulation comprising an aqueous carrier liquid having dissolved therein (a) an ester of fluticasone or a solvate thereof as medicament and (b) a solubilising agent for assisting the solubilisation of the medicament in the aqueous carrier liquid.

FORMULATION CONTAINING ANTI-INFLAMMATORY ANDROSTANE DERIVATIVES

[0001] This application is a Continuation-in-part of a US 35 USC 371, Ser. No. 09/958050 filed on Oct. 2, 2001 in the United States Patent and Trademark Office, for which an International Patent Application, PCT.GB01.03495 was filed Aug. 3, 2001, which claims priority to United Kingdom Patent Application No. GB 0019172.6 filed Aug. 5, 2000.

[0002] The present invention relates to a pharmaceutical formulations containing anti-inflammatory and anti-allergic compound of the androstanes series and to processes for their preparation. The present invention also relates to therapeutic uses thereof, particularly for the treatment of inflammatory and allergic conditions.

[0003] Glucocorticoids which have anti-inflammatory properties are known and are widely used for the treatment of inflammatory disorders or diseases such as asthma and rhinitis. For example, U.S. Pat. No. 4,335,121 discloses 6α, 9α-Difluoro-17α-(1-oxopropoxy)-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid S-fluoromethyl ester (known by the generic name of fluticasone propionate) and derivatives thereof. The use of glucocorticoids generally, and especially in children, has been limited in some quarters by concerns over potential side effects. The side effects that are feared with glucocorticoids include suppression of the Hypothalamic-Pituitary-Adrenal (HPA) axis, effects on bone growth in children and on bone density in the elderly, ocular complications (cataract formation and glaucoma) and skin atrophy. Certain glucocorticoid compounds also have complex paths of metabolism wherein the production of active metabolites may make the pharmacodynamics and pharmacokinetics of such compounds difficult to understand. Whilst the modern glucocorticoids are very much safer than those originally introduced, it remains an object of research to produce new molecules and formulations of old and new molecules which have excellent anti-inflammatory properties, with predictable pharmacokinetic and pharmacodynamic properties, with an attractive side effect profile, and with a convenient treatment regime.

[0004] We have now identified a novel glucocorticoid compound and formulation thereof which substantially meets these objectives. In particular we have invented a novel glucocorticoid compound and other esters of fluticasone including fluticasone propionate.

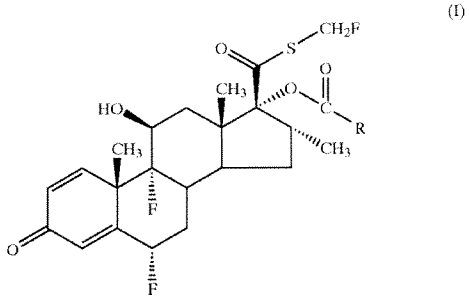
[0005] Many millions of individuals suffer from seasonal and perennial allergic rhinitis worldwide. Symptoms of seasonal and perennial allergic rhinitis include nasal itch, congestion, runny nose, sneezing and watery eyes. Seasonal allergic rhinitis is commonly known as 'hay fever'. It is caused by allergens which are present in the air at specific times of the year, for example tree pollen during Spring and Summer. Perennial allergic rhinitis is caused by allergens which are present in the environment during the entire year, for example dust mites, mold, mildew and pet dander.

[0006] To formulate an effective pharmaceutical nasal composition, the medicament must be delivered readily to all portions of the nasal cavities (the target tissues) where it performs its pharmacological function. Additionally, the medicament should remain in contact with the target tissues for relatively long periods of time. The longer the medicament remains in contact with the target tissues, the medicament must be capable of resisting those forces in the nasal passages that function to remove particles from the nose. Such forces, referred to as 'mucociliary clearance', are recognised as being extremely effective in removing particles from the nose in a rapid manner, for example, within 10-30 minutes from the time the particles enter the nose.

[0007] Other desired characteristics of a nasal composition are that it must not contain ingredients which cause the user discomfort, that it has satisfactory stability and shelf-life properties, and that it does not include constituents that are considered to be detrimental to the environment, for example ozone depletors. In the case of administration of glucocorticoids, the potential for any undesirable side effects should preferably be minimised.

[0008] Thus, according to one aspect of the invention, there is provided a pharmaceutical formulation comprising an aqueous carrier liquid having dissolved therein (a) an ester of fluticasone or a solvate thereof as medicament and (b) a solubilising agent for assisting the solubilisation of the medicament in the aqueous carrier liquid.

[0009] Preferably the ester of fluticasone is a compound of formula (I)



[0010] wherein R represents ethyl or a 5 membered heterocyclic aromatic ring containing 1 to 3 heteroatoms selected from O, N and S, optionally substituted by one or more methyl or halogen atoms or a solvate thereof.

[0011] Solvates of esters of fluticasone includes solvates with pharmaceutically acceptable solvents eg hydrates.

[0012] In a first embodiment of the invention, preferably R represents ethyl and the compound of formula (I) is fluticasone propionate.

[0013] In a second embodiment of the process, preferably R represents furan-2-yl and the compound of formula (I) has the chemical name: 6α, 9α-difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid S-fluoromethyl ester.

[0014] Hitherto, nasal formulations of glucocorticoid compounds, particularly aqueous formulations of glucocorticoid compounds, have been in the form of suspensions. In such suspension products the active ingredient is suspended in the aqueous carrier in the form of finely divided particles, typically of mass median diameter (MMD) 1-5 microns. Particles of this size are typically produced by micronisation, which is a wasteful and hazardous process.

[0015] Solution formulations have advantages in that the use of micronisation processes may be avoided and also in that onset of action may be increased since it is not necessary for any dissolution process to take place before the drug enters the cells in which it acts and exerts its anti-inflammatory effect. However fluticasone esters are quite insoluble in water (typically less than 1 μg/ml) and so it might be thought that the large volumes of dilute liquid which would need to be administered to have therapeutic effect would be impractical. We have now surprisingly discovered that the presence of a solubilising agent which is preferably a surfactant, especially a surfactant selected from the group consisting of a α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly(oxy-1,2-ethanediyl) polymer (also known as a octylphenoxypolyethoxyethanol) and a 4-(1,1,3,3-tetramethylbutyl)phenol polymer with formaldehyde and oxirane significantly increases the solubility of fluticasone esters in water thus permitting acceptably concentrated solutions to be developed. The solubility of fluticasone esters in water in the presence of such a surfactant is maximised when the formulation is prepared in a particular manner as described later which forms a particular aspect of the invention.

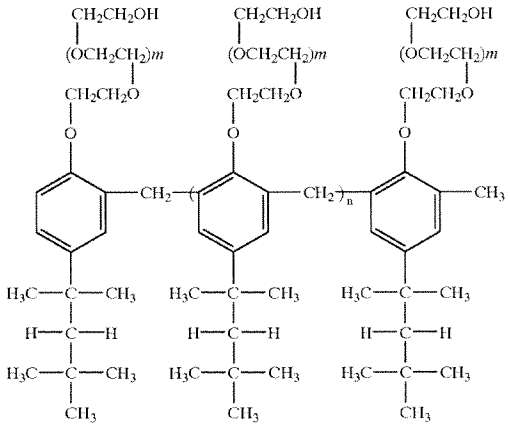
[0016] We have also surprisingly discovered that the solubility of fluticasone esters can be increased yet further by dissolution in the aqueous carrier liquid of a hydroxyl containing organic co-solvating agent or of phosphatidiyl choline. Thus formulations including this additional component have further advantages and are preferred.

[0017] Examples of α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly(oxy-1,2-ethanediyl) polymer surfactants include those of the Triton series eg Triton X-100, Triton X-114 and Triton X-305 in which the X number is broadly indicative of the average number of ethoxy repeating units in the polymer. For example the average number of ethoxy repeating units in a series of Triton X surfactants is as follows:

Triton	Average number of ethoxy units
X-45	5
X-114	7–8
X-100	9–10
X-102	12–13
X-165	16
X-305	30
X-405	40
X-705	70

[0018] Preferably the number of repeating units in the α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly-(oxy-1,2-ethanediyl) polymer is around 7-70, particularly around 7-30 especially around 7-10.

[0019] 4-(1,1,3,3-Tetramethylbutyl)phenol polymers with formaldehyde and oxiran typically have a relative molecular weight of 3500-5000 especially 4000-4700. An example structure is given below:



m = 6–8

n ≤ 5

For m = 7 and n = 5, MW is 4,326

[0020] Thus as just noted, m may represent 6-8 eg 7 and n may represent 1-5, especially 3-5 eg 5. The preferred example is Tyloxapol.

[0021] Preferably the surfactant is Triton X-100 or Tyloxapol since these surfactants have the highest solubilising power and can therefore be employed at the lowest concentrations. The most preferred surfactant is Tyloxapol.

[0022] The solubility of fluticasone propionate in various surfactants in water with and without an organic co-solvating agent or phosphatidyl choline is given in the following table:

Surfactant	Concentration (% w/w)	Solubility (μg/ml)
Triton X-100	2	69
Tyloxapol	2	41
Tyloxapol	5	133
Tyloxapol and	5	197
Phospatidyl choline	1	
Tyloxapol and	5	207
PEG200	10	
Labrasol	10	None detected
Sodium chenodesoxycholate	5	5.9
Triton X-45	5	None detected
Sodium cholate	20	140
	2	11
—	—	<1

[0023] Labrasol, sodium chenodesoxycholate, Triton X-45 and sodium cholate were not considered suitable for use in formulations according to the invention since excessively high concentrations of such surfactants are needed to be used to dissolve the fluticasone ester to a sufficient extent.

[0024] The solubility in water of compound of formula (I) in which R represents furan-2-yl in various surfactants is shown in the following table:

Surfactant	Concentration (% w/w)	Solubility (μg/ml)
Triton X-100	0.5	18
"	2	266
"	5	549
Triton X-100 and PEG200	5 10	840
Tyloxapol	2	95
"	5	307
"	6	346
"	7.5	576
"	10	585
Triton X-305	5	204
—	—	<1

[0025] In formulations of the invention, the surfactant will typically be employed in a concentration of around 0.5-10%, preferably around 2-5% w/w based on weight of formulation. The precise concentration chosen will depend on the nature and concentration of the glucorticoid. The surfactant will need to be soluble in the formulation at the concentration used.

[0026] Examples of hydroxyl containing organic co-solvating agents include glycols such as polyethylene glycols (eg PEG 200) and propylene glycol; sugars such as dextrose; and ethanol. Dextrose and polyethylene glycol (eg PEG 200) are preferred, particularly dextrose. Propylene glycol is preferably used in an amount of no more than 20%, especially no more than 10% and is most preferably avoided altogether. Ethanol is also preferably avoided.

[0027] The hydroxyl containing organic co-solvating agents are typically employed at a concentration of 1-20% eg 5-10%, eg around 5% w/w based on weight of formulation. When phosphatidyl choline is employed it is typically employed at a concentration of 0.1-10% eg 0.5-5%, eg around 1% w/w based on weight of formulation.

[0028] The effect of addition of a hydroxyl containing organic co-solvating agent to solubility in water of compound of formula (I) in which R represents furan-2-yl in various surfactants is shown in the following table:

Surfactant/co-solvating agent	Concentration (% w/w)	Solubility (μg/ml)
Tyloxapol	5	344
Tyloxapol	5	836
PEG200	10	
Tyloxapol	5	422
Propylene glycol	10	
Tyloxapol	5	526
Dextrose	4	

[0029] The aqueous carrier liquid will essentially comprise water.

[0030] However for nasal administration it may also have dissolved in it one or more of the following components:

[0031] viscosity enhancing agents.

[0032] preservatives.

[0033] isotonicity adjusting agents.

[0034] The formulations of the present invention may be stabilised by appropriate selection of pH using hydrochloric acid. Typically, the pH will be adjusted to between 4.5 and 7.5, preferably between 5.0 and 7.0, especially around 6.5.

[0035] Examples of pharmaceutically acceptable materials which can be used to adjust the pH of the formulation include hydrochloric acid and sodium hydroxide. Preferably, the pH of the formulation will be adjusted using hydrochloric acid.

[0036] The aqueous component is preferably a high grade quality of water, most preferably purified water.

[0037] Examples of viscosity enhancing agents include carboxymethylcellulose, veegum, tragacanth, bentonite, hydroxypropylmethylcellulose, hydroxypropylcellulose, hydroxyethylcellulose, poloxamers (eg. poloxamer 407), polyethylene glycols, alginates xanthym gums, carageenans and carbopols. Preferably, the viscosity enhancing agent will be carboxy methylcellulose sodium.

[0038] Preferably, the viscosity enhancing agent will possess thixotropic properties which will ensure that the formulation assumes a gel like appearance at rest, characterised by a high viscosity value. Once the composition is subjected to shear forces, such as those caused by agitation prior to spraying, the viscosity of the formulation will preferably decrease transiently to such a level to enable it to flow readily through the spray device and exit as a fine mist spray. This mist will then be capable of infiltrating the mucosal surfaces of the anterior regions of the nose (frontal nasal cavities), the frontal sinus, the maxillary sinuses and the turbinates which overlie the conchas of the nasal cavities. Once deposited, the viscosity of the formulation will preferably increase to a sufficient level to assume its gel-like form and resist being cleared from the nasal passages by the inherent mucocillary forces that are present in the nasal cavities.

[0039] When the formulation of the present invention comprises a viscosity enhancing agent, it will be desirably added in a suitable amount to achieve this function, preferably the viscosity enhancing agent will be present within the formulation in an amount of between 0.1 and 5% (w/w), eg 1.5% (w/w), based on the total weight of the formulation.

[0040] For stability purposes, the formulation of the present invention should be protected from microbial contamination and growth. Examples of pharmaceutically acceptable anti-microbial agents that can be used in the formulation include quaternary ammonium compounds (eg. benzalkonium chloride, benzethonium chloride, cetrimide and cetylpyridinium chloride), mercurial agents (eg. phenylmercuric nitrate, phenylmercuric acetate and thimerosal), alcoholic agents (eg. chlorobutanol, phenylethyl alcohol and benzyl alcohol), antibacterial esters (eg. esters of para-hydroxybenzoic acid), chelating agents such as disodium edetate (EDTA) and other anti-microbial agents such as chlorhexidine, chlorocresol, sorbic acid and its salts and polymyxin.

[0041] Preferably, the preservative will comprise disodium edetate, which will preferably be present within the formulation in an amount of between 0.001 and 1% (w/w), especially around 0.015% (w/w), based on the total weight of the formulation.

[0042] Preferably the preservative will comprise potassium sorbate which will preferably be present within the formultaion in an amount of between 0.01 and 1% (w/w), especially around 0.015% (w/w) based on the total weight of the formulation.

[0043] Preferably, the preservative will comprise benzalkonium chloride (BKC), which will preferably be present within the formulation in an amount of between 0.001 and 1% (w/w), especially around 0.015% (w/w), based on the total weight of the formulation.

[0044] More preferably, the preservative comprises disodium edetate and benzalkonium chloride.

[0045] The presence of an isotonicity adjusting agent is to achieve isotonicity with body fluids eg fluids of the nasal cavity, resulting in reduced levels of irritancy associated with many nasal formulations. Examples of suitable isotonicity adjusting agents are sodium chloride, dextrose and calcium chloride. Preferably, the isotonicity adjusting agent will be dextrose, most preferably used as dextrose anhydrous.

[0046] When the formulation of the present invention comprises an isotouicity adjusting agent it will be desirably added in a sufficient quantity to achieve this function, preferably the isotonicity adjusting agent will be present within the formulation in an amount of between 0.1 and 10% (w/w), especially 5.0% w/w, based on the total weight of the formulation.

[0047] Fluticasone esters eg the compounds of formula (I), especially when R represents furan-2-yl, and formulations thereof have potentially beneficial anti-inflammatory or anti-allergic effects, particularly upon topical administration to the nose, demonstrated by, for example, its ability to bind to the glucocorticoid receptor and to illicit a response via that receptor, with long acting effect. Hence, formulations according to the invention are useful in the treatment of inflammatory and/or allergic disorders of the nose, especially in once-per-day therapy.

[0048] Formulations according to the invention may be prepared by dissolving the ingredients in water. If necessary the pH may be adjusted as a final step. Generally it will be desirable to filter the solution to remove any residual particulate matter. Formulations so prepared may then be filled into the receptacle.

[0049] We have however invented an improved process for preparing formulations according to the invention which comprises:

[0050] (a) dissolving the fluticasone ester in the undiluted surfactant at an elevated temperature (typically 60-70° C.);

[0051] (b) adding hot water (typically 60-70° C.) together with other formulation ingredients to achieve the desired concentration of active ingredient;

[0052] (c) if desired modifying the pH of the final solution;

[0053] (d) filtering the hot solution to remove any residual particulate matter.

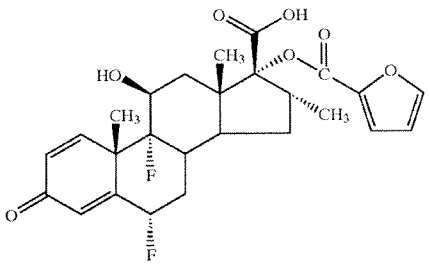
[0054] Preferably the temperature of the liquids in steps (a) and (b) and the temperature of the liquid filtered in step (c) is 45° C. or greater, preferably 55° C. or greater, especially 65° C. or greater. The temperature is preferably 800° C. or less, eg 70° C. or less.

[0055] As noted above, this improved process permits solutions of higher concentration to be prepared than is possible by conventional techniques. For example this process performed at 60-70° C. increases the solubility of fluticasone propionate from 87 to 133 μg/ml in 5% Tyloxapol and the compound of of formula (I) when R represents furan-2-yl from 233 to 344 μg/ml in 5% Tyloxapol relative to the process performed at room temperature.

[0056] Aqueous formulations of the invention may also be employed for rectal, aural, otic, oral, topical or parenteral administration or administration by inhalation for the treatment of other local inflammatory conditions (eg dermatitis, asthma, chronic obstructive pulmonary disease (COPD) and the like). For example formulations of the invention may be administered to the lung by nebulisation. Such formulations may employ excipients (eg preservatives, buffers and the like) appropriate for the route of administration.

[0057] The particularly desirable biological properties of the compound of formula (I) wherein R represents furan-2-yl are now explained below: Compound (I) wherein R represents furan-2-yl undergoes highly efficient hepatic metabolism to yield the 17-β carboxylic acid (X) as the sole major metabolite in rat and human in vitro systems. This metabolite has been synthesised and demonstrated to be >1000 fold less active than the parent compound in in vitro functional glucocorticoid assays.

(X)



[0058] This efficient hepatic metabolism is reflected by in vivo data in the rat, which have demonstrated plasma clearance at a rate approaching hepatic blood flow and an oral bioavailability of <1%, consistent with extensive first-pass metabolism.

[0059] In vitro metabolism studies in human hepatocytes have demonstrated that compound (I) is metabolised in an identical manner to fluticasone propionate but that conversion of (I) to the inactive acid metabolite occurs approximately 5-fold more rapidly than with fluticasone propionate. This very efficient hepatic inactivation would be expected to minimise systemic exposure in man leading to an improved safety profile.

[0060] Inhaled glucocorticoids are also absorbed through the lung and this route of absorption makes a significant contribution to systemic exposure. Reduced lung absorption

could therefore provide an improved safety profile. Studies with compound (I) have shown significantly lower exposure to compound (I) than with fluticasone propionate after dry powder delivery to the lungs of anaesthetised pigs.

[0061] Examples of disease states in which fluticasone esters have utility include inflammatory and/or allergic conditions of the nasal passages such as rhinitis eg seasonal and perennial rhinitis as well as other local inflammatory conditions such as asthma, COPD and dermatitis.

[0062] It will be appreciated by those skilled in the art that reference herein to treatment extends to prophylaxis as well as the treatment of established conditions.

[0063] Preferable means for applying the formulation of the present invention to the nasal passages is by use of a pre-compression pump. Most preferably, the pre-compression pump will be a VP7 model manufactured by Valois SA. Such a pump is beneficial as it will ensure that the formulation is not released until a sufficient force has been applied, otherwise smaller doses may be applied. Another advantage of the pre-compression pump is that atomisation of the spray is ensured as it will not release the formulation until the threshold pressure for effectively atomising the spray has been achieved. Typically, the VP7 model may be used with a bottle capable of holding 10-50 ml of a formulation. Each spray will typically deliver 50-100 μl of such a formulation, therefore, the VP7 model is capable of providing at least 100 metered doses.

[0064] A suitable dosing regime for the formulation of the present invention when administered to the nose would be for the patient to inhale deeply subsequent to the nasal cavity being cleared. During inhalation the formulation would be applied to one nostril while the other is manually compressed. This procedure would then be repeated for the other nostril.

[0065] Typically, one or two inhalations per nostril would be administered by the above procedure up to three times each day, preferably once or twice daily especially once daily.

[0066] It will be appreciated that the above dosing regime should be adjusted according to the patient's age, body weight and/or symptom severity.

[0067] As mentioned above, formulations comprising a fluticasone esters are useful in human or veterinary medicine, in particular as an anti-inflammatory and anti-allergic agent.

[0068] There is thus provided as a further aspect of the invention a formulation comprising the fluticasone ester or a physiologically acceptable solvate thereof for use in human or veterinary medicine, particularly in the treatment of patients with inflammatory and/or allergic conditions.

[0069] According to another aspect of the invention, there is provided the use of a formulation comprising the fluticasone ester or physiologically acceptable solvate thereof for the manufacture of a medicament for the treatment of patients with inflammatory and/or allergic conditions.

[0070] In a further or alternative aspect, there is provided a method for the treatment of a human or animal subject with an inflammatory and/or allergic condition, which method comprises administering to said human or animal subject an effective amount of a formulation comprising the fluticasone ester or physiologically acceptable solvate thereof.

[0071] Further, there is provided a process for the preparation of such pharmaceutical compositions which comprises mixing the ingredients.

[0072] The proportion of the active fluticasone ester in the local compositions according to the invention depends on the precise type of formulation to be prepared but will generally be within the range of around 0.001-12%, more preferably 0.001 to 10% by weight. Generally, however for most types of preparations advantageously the proportion used will be within the range of from 0.001 to 1%, more preferably 0.001-0.5, and especially around 0.005 to 0.1%.

[0073] The compound of formula (I) wherein R represents furan-2-yl is long-acting, therefore preferably the compound will be delivered once-per-day and the dose will be selected so that the compound has a therapeutic effect in the treatment of respiratory disorders (eg rhinitis) over 24 hours or more.

[0074] The pharmaceutical compositions according to the invention may also be used in combination with another therapeutically active agent, for example, an anti-histamine or an anti-allergic. The invention thus provides, in a further aspect, a combination comprising the fluticasone ester or a physiologically acceptable solvate thereof together with another therapeutically active agent, for example, an anti-histamine or an anti-allergic.

[0075] Examples of anti-histamines include methapyrilene or loratadine.

[0076] Other suitable combinations include, for example, other anti-inflammatory agents eg NSAIDs (eg sodium cromoglycate, nedocromil sodium, PDE4 inhibitors, leukotriene antagonists, iNOS inhibitors, tryptase and elastase inhibitors, beta-2 integrin antagonists and adenosine 2a agonists)) or antiinfective agent (eg antibiotics, antivirals).

[0077] Also of particular interest is use of the fluticasone ester or a physiologically acceptable solvate thereof in combination with a phosphodiesterase 4 (PDE4) inhibitor eg cilomilast or a salt thereof.

[0078] For administration to the lung, the pharmaceutical compositions according to the invention may also be used in combination with a β_2 adrenoreceptor agonist. Examples of β_2-adrenoreceptor agonists include salmeterol (eg as racemate or single enantiomer such as the s-enantiomer), salbutamol, formoterol, fenoterol or terbutaline and salts thereof, for example the xinafoate salt of salmeterol, the sulphate salt or free base of salbutamol or the fumarate salt of formoterol. Long-acting $_2$-adrenoreceptor agonists are preferred, especially those having a therapeutic effect over a 24 hour period.

[0079] Since the compounds of formula (I) are long-acting, preferably a composition comprising the compound of formula (I) and the long-acting β_2-adrenoreceptor agonists will be delivered once-per-day and the dose of each will be selected so that the composition has a therapeutic effect in the treatment of respiratory disorders effect (eg in the treatment of asthma or COPD, particularly asthma) over 24 hours or more.

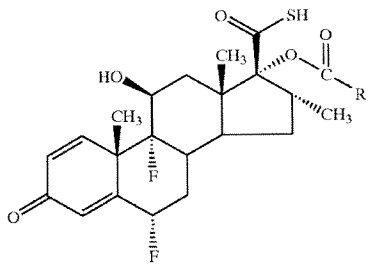
[0080] Further, there is provided a process for the preparation of such pharmaceutical compositions which comprises mixing the ingredients.

[0081] The individual compounds of such combinations may be administered either sequentially in separate pharmaceutical compositions as well as simultaneously in combined pharmaceutical formulations. Preferably additional therapeutically active ingredients are dissolved in the formulation together with the fluticasone ester. Appropriate doses of known therapeutic agents will be readily appreciated by those skilled in the art.

[0082] Fluticasone esters may generally be prepared following the methods of GB2088877B and Phillips et al (1994) J Med Chem, 37, 3717-3729.

[0083] For example, a process for preparing a compound of formula (I) and other fluticasone esters comprises alkylation of a thioacid of formula (II)

(II)



[0084] or a salt thereof.

[0085] In this process the compound of formula (II) may be reacted with a compound of formula FCH_2L wherein L represents a leaving group (eg a halogen atom, a mesyl or tosyl group or the like) for example, an appropriate fluoromethyl halide under standard conditions. Preferably, the fluoromethyl halide reagent is bromofluoromethane. Preferably the compound of formula (II) is employed as a salt, particularly the salt with diisopropylethylamine.

[0086] In a preferred process for preparing the compound of formula (I), the compound of formula (II) or a salt thereof is treated with bromofluoromethane optionally in the presence of a phase transfer catalyst. A preferred solvent is methylacetate, or more preferably ethylacetate, optionally in the presence of water. The presence of water improves solubility of both starting material and product and the use of a phase transfer catalyst results in an increased rate of reaction. Examples of phase transfer catalysts that may be employed include (but are not restricted to) tetrabutylammonium bromide, tetrabutylammonium chloride, benzyltributylammonium bromide, benzyltributylammonium chloride, benzyltriethylammonium bromide, methyltributylammonium chloride and methyltrioctylammonium chloride. THF has also successfully been employed as solvent for the reaction wherein the presence of a phase transfer catalyst again provides a significantly faster reaction rate. Preferably the product present in an organic phase is washed firstly with aqueous acid eg dilute HCl in order to remove amine compounds such as triethylamine and diisopropylethylamine and then with aqueous base eg sodium bicarbonate in order to remove any unreacted precursor compound of formula (II).

[0087] Compound of formula (I) wherein R represents furan-2-yl in unsolvated form may be prepared by a process comprising:

[0088] (a) Crystallising the compound of formula (I) in the presence of a non-solvating solvent such as ethanol, methanol, water, ethyl acetate, toluene, methylisobutylketone or mixtures thereof; or

[0089] (b) Desolvating a compound of formula (I) in solvated form (eg in the form of a solvate with acetone, isopropanol, methylethylketone, DMF or tetrahydrofuran) eg by heating.

[0090] In-step (D) the desolvation will generally be performed at a temperature exceeding 50° C. preferably at a temperature exceeding 100° C. Generally heating will be performed under vacuum.

[0091] Compound of formula (I) wherein R represents furan-2-yl has been prepared in three crystalline polymorphic forms designated Form 1, Form 2 and Form 3 which are distinguishable by their X-ray powder diffraction (XRPD) profiles. Form 3 appears to be an unstable variant of Form 2. Form 1 appears to be the thermodynamically most stable form and is therefore preferred.

[0092] A process for preparing a compound of formula (I) as unsolvated Form 1 polymorph comprises dissolving compound of formula (I) in methylisobutylketone, ethyl acetate or methyl acetate and producing compound of formula (I) as unsolvated Form 1 by addition of a non-solvating anti-solvent such as iso-octane or toluene.

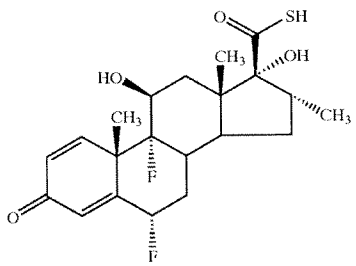
[0093] According to a first preferred embodiment of this process the compound of formula (1) may be dissolved in ethyl acetate and compound of formula (I) as unsolvated Form 1 polymorph may be obtained by addition of toluene as anti-solvent. In order to improve the yield, preferably the ethyl acetate solution is hot and once the toluene has been added the mixture is distilled to reduce the content of ethyl acetate.

[0094] According to a second preferred embodiment of this process the compound of formula (I) may be dissolved in methylisobutylketone and compound of formula (I) as unsolvated Form 1 polymorph may be obtained by addition of isooctane as anti-solvent

[0095] Compound of formula (I) in solvated form may be prepared by crystallising the compound of formula (I) from a solvating solvent such as acetone or tetrahydrofuran (THF).

[0096] Compounds of formula (II) may be prepared from the corresponding 17α-hydroxyl derivative of formula (III):

(III)



[0097] using for example, the methodology described by G. H. Phillipps et al., (1994) Journal of Medicinal Chemistry, 37, 3717-3729. For example the step typically comprises the addition of a reagent suitable for performing the esterification eg an activated derivative of RCOOH such as an activated ester or preferably an acid halide eg RCOOCl (employed in at least 2 times molar quantity relative to the compound of formula (III)) in the presence of an organic base eg triethylamine. The second mole of RCOOCl reacts with the thioacid moiety in the compound of formula (III) and needs to be removed eg by reaction with an amine such as diethylamine.

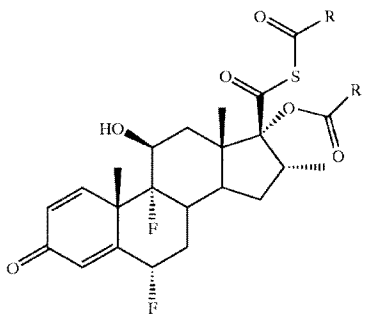
[0098] This method suffers disadvantages, however, in that the resultant compound of formula (II) is not readily purified of contamination with the by-product $RCON(Et)_2$ We have therefore invented several improved processes for performing this conversion.

[0099] In a first such improved process we have discovered that by using a more polar amine such as diethanolamine, a more water soluble by-product is obtained (in this case RCO-diethanolamide) which permits compound of formula (II) or a salt thereof to be produced in high purity since the by-product can efficiently be removed by water washing.

[0100] A process for preparing a compound of formula (II) comprises:

[0101] (a) reacting a compound of formula (III) with an activated derivative of RCOOH as in an amount of at least 2 moles of the activated derivative per mole of compound of formula (III) to yield a compound of formula (IIA)

(IIA)



[0102] ; and

[0103] (b) removal of the sulphur-linked R—CO— moiety from compound of formula (IIA) by reaction of the product of step (a) with an organic primary or secondary amine base capable of forming a water soluble amide.

[0104] In two particularly convenient embodiments of this process we also provide methods for the efficient purification of the end product which comprise either

[0105] (c1)when the product of step (b) is dissolved in a substantially water immiscible organic solvent, purifying the compound of formula (II) by washing out the amide by-product from step (b) with an aqueous wash, or

[0106] (c2) when the product of step (b) is dissolved in a water miscible solvent, purifying the compound of formula (II) by treating the product of step (b) with an aqueous medium so as to precipitate out pure compound of formula (II) or a salt thereof.

[0107] In step (a) preferably the activated derivative of RCOOH may be an activated ester of RCOOH, but is more preferably an acid halide, especially RCOOCl. A suitable solvent for this reaction is ethylacetate or methylacetate (preferably methylacetate) (when step (c1) may be followed) or acetone (when step (c2) may be followed). Normally an organic base eg triethylamine will be present. In step (b) preferably the organic base is diethanolamine. The base may suitably be dissolved in a solvent eg methanol. Generally steps (a) and (b) will be performed at reduced temperature eg between 0 and 5° C. In step (c1) the aqueous wash may be water, however the use of brine results in higher yields and is therefore preferred. In step (c2) the aqueous medium is for example a dilute aqueous acid such as dilute HCl.

[0108] An alternative process for preparing a compound of formula (II) comprises:

[0109] (a) reacting a compound of formula (III) with an activated derivative of RCOOH in an amount of at least 2 moles of activated derivative per mole of compound of formula (III) to yield a compound of formula (IIA); and

[0110] (b) removal of the sulphur-linked RCO moiety from compound of formula (IIA) by reaction of the product of step (a) with a further mole of compound of formula (III) to give two moles of compound of formula (II).

[0111] In step (a) preferably the activated derivative of RCOOH may be an activated ester of RCOOH, but is more preferably an acid halide, especially RCOOCl. A suitable solvent for his step is acetone. Normally an organic base eg triethylamine will be present, In step (b) a suitable solvent is DMF or dimethylacetamide. Normally an organic base eg triethylamine will be present. Generally steps (a) and (b) will be performed at reduced temperature eg between 0 and 5° C. The product may be isolated by treatment with acid and washing with water.

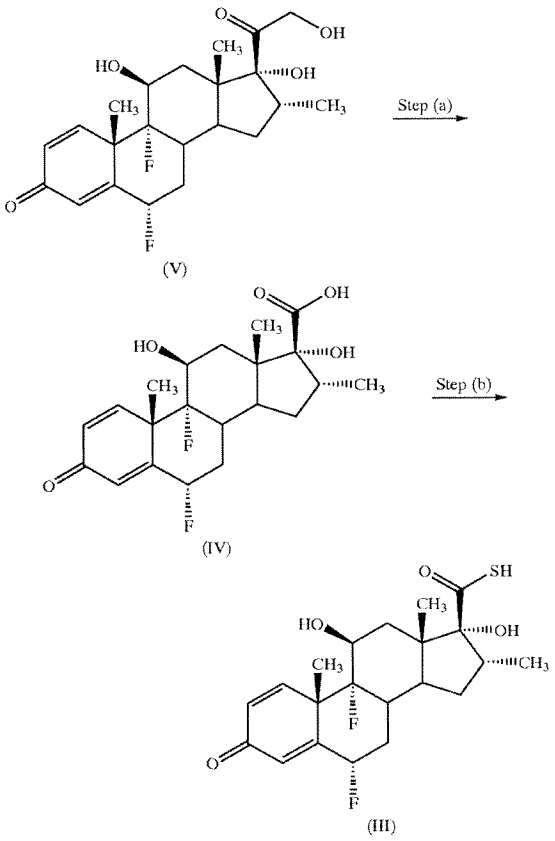
[0112] This aforementioned process is very efficient in that it does not produce any amide by-product (thus affording inter alia environmental advantages) since the excess mole of ester moiety is taken up by reaction with a further mole of compound of formula (II) to form an additional mole of compound of formula (II).

[0113] Further general conditions for the conversion of compound of formula (III) to compound of formula (II) in the two processes just described will be well known to persons skilled in the art.

[0114] We have found that the compound of formula (II) may advantageously be isolated in the form of a solid crystalline salt. The preferred salt is a salt formed with a base such as triethylamine, 2,4,6-trimethylpyridine, diisopropylethylamine or N-ethylpiperidine. Such salt forms of compound of formula (II) are more stable, more readily filtered and dried and can be isolated in higher purity than the free thioacid. The most preferred salt is the salt formed with diisopropylethylamine. The triethylamine salt is also of interest.

[0115] Compounds of formula (III) may be prepared in accordance with procedures described in GB 2088877B.

[0116] Compounds of formula (III) may also be prepared by a process comprising the following steps:



[0117] Step (a) comprises oxidation of a solution containing the compound of formula (V). Preferably, step (a) will be performed in the presence of a solvent comprising methanol, water, tetrahydrofuran, dioxan or diethylene glygol dimethylether. So as to enhance yield and throughput, preferred solvents are methanol, water or tetrahydrofuran, and more preferably are water or tetrahydrofuran especially water and tetrahydrofuran as solvent. Dioxan and diethylene glygol dimethylether are also preferred solvents which may optionally (and preferably) be employed together with water. Preferably, the solvent will be present in an amount of between 3 and 10 vol relative to the amount of the starting material (1 wt.), more preferably between 4 and 6 vol., especially 5 vol. Preferably the oxidising agent is present in an amount of 1-9 molar equivalents relative to the amount of the starting material. For example, when a 50% w/w aqueous solution of periodic acid is employed, the oxidising agent may be present in an amount of between 1.1 and 10 wt. relative to the amount of the starting material (1 wt.), more preferably between 1.1 and 3 wt., especially 1.3 wt. Preferably, the oxidation step will comprise the use of a chemical oxidising agent. More preferably, the oxidising agent will be periodic acid or iodic acid or a salt thereof. Most preferably, the oxidising agent will be periodic acid or sodium periodate, especially periodic acid. Alternatively (or in addition), it will also be appreciated that the oxidation step may comprise any suitable oxidation reaction, eg one which utilises air and/or oxygen. When the oxidation reaction utilises air and/or oxygen, the solvent used in said reaction will preferably be methanol. Preferably, step (a) will involve incubating the reagents at room temperature or a little warmer, say around 25° C. eg for 2 hours. The compound of formula (IV) may be isolated by recrystallisation from the reaction mixture by addition of an anti-solvent. A suitable anti-solvent for compound of formula (IV) is water. Surprisingly we have discovered that it is highly desirable to control the conditions under which the compound of formula (IV) is precipitated by addition of anti-solvent eg water. When the recrystallisation is performed using chilled water (eg water/ice mixture at a temperature of 0-5° C.) although better anti-solvent properties may be expected we have found that the crystalline product produced is very voluminous, resembles a soft gel and is very difficult to filter. Without being limited by theory we believe that this low density product contains a large amount of solvated solvent within the crystal lattice. By contrast when conditions of around 10° C. or higher are used (eg around ambient temperature) a granular product of a sand like consistency which is very easily filtered is produced. Under these conditions, crystallisation typically commences after around 1 hour and is typically completed within a few hours (eg 2 hours). Without being limited by theory we believe that this granular product contains little or no solvated solvent within the crystal lattice.

[0118] Step (b) will typically comprise the addition of a reagent suitable for converting a carboxylic acid to a carbothioic acid eg using hydrogen sulphide gas together with a suitable coupling agent eg carbonyldiimidazole (CDI) in the presence of a suitable solvent eg dimethylformamide.

[0119] The advantages of the formulation of the fluticasone esters according to the invention may include the fact that the formulations demonstrate excellent anti-inflammatory properties, with predictable pharmacokinetic and pharmacodynamic behaviour, with an attractive side-effect profile, rapid onset of action, long duration of action, and are compatible with a convenient regime of treatment in human patients, in particular being amendable to once-per day dosing. Further advantages may include the fact that the formulation has desirable physical and chemical properties which allow for ready manufacture and storage.

[0120] The following non-limiting Examples illustrate the invention:

EXAMPLES

General

[0121] ^{1}H-nmr spectra were recorded at 400 MHz and the chemical shifts are expressed in ppm relative to tetramethylsilane. The following abbreviations are used to describe the multiplicities of the signals: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets), ddd (doublet of doublet of doublets), dt (doublet of triplets) and b (broad). Biotage refers to prepacked silica gel cartridges containing KP-Sil run on flash 12i chromatography module. LCMS was conducted on a Supelcosil LCABZ+

PLUS column (3.3 cm×4.6 mm ID) eluting with 0.1% HCO_2H and 0.01 M ammonium acetate in water (solvent A), and 0.05% HCO_2H 5% water in acetonitrile (solvent B), using the following elution gradient 0-0.7 min 0%B, 0.7-4.2 min 100%B, 4.2-5.3 min 0%B, 5.3-5.5 min 0%B at a flow rate of 3 ml/min. The mass spectra were recorded on a Fisons VG Platform spectrometer using electrospray positive and negative mode (ES+ve and ES−ve).

Intermediates

Intermediate 1: 6α, 9α-Difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid diisopropylethylamine salt

[0122] A stirred suspension of 6α, 9α-difluoro-11β, 17α-dihydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid (prepared in accordance with the procedure described in GB 2088877B) (49.5 g) in methylacetate (500 ml) is treated with triethylamine (35 ml) maintaining a reaction temperature in the range 0-5° C. 2-Furoyl chloride (25 ml) is added and the mixture stirred at 0-50° C. for 1 hour. A solution of diethanolamine (52.8 g) in methanol (50 ml) is added and the mixture stirred at 0-5° C. for at least 2 hours. Dilute hydrochloric acid (approx 1M, 550 ml) is added maintaining a reaction temperature below 150° C. and the mixture stirred at 15° C. The organic phase is separated and the aqueous phase is back extracted with methyl acetate (2×250 ml). All of the organic phases are combined, washed sequentially with brine (5×250 ml) and treated with diisopropylethylamine (30 ml). The reaction mixture is concentrated by distillation at atmospheric pressure to an approximate volume of 250 ml and cooled to 25-30° C. (crystallisation of the desired product normally occurs during distillation/subsequent cooling). Tertiary butyl methyl ether (TBME) (500 ml) is added, the slurry further cooled and aged at 0-5° C. for at least 10 minutes. The product is filtered off, washed with chilled TBME (2×200 ml) and dried under vacuum at approximately 40-50° C. (75.3 g, 98.7%). NMR ($CDCl_3$) δ: 7.54-7.46 (1H, m), 7.20-7.12 (1H, dd), 7.07-6.99 (1H, dd), 6.48-6.41 (2H, m), 6.41-6.32 (1H, dd), 5.51-5.28 (1H, dddd $^2J_{H-F}$ 50 Hz), 4.45-4.33(1 H, bd), 3.92-3.73 (3H, bm), 3.27-3.14 (2H, q), 2.64-2.12 (5H, m), 1.88-1.71 (2H, m), 1.58-1.15 (3H, s), 1.50-1.38 (15H, m), 1.32-1.23 (1H, m), 1.23-1.15 (3H s), 1.09-0.99 (3H, d)

Intermediate 2: 6α, 9α-Difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid S-fluoromethyl ester Unsolvated Form 1

[0123] A mobile suspension of Intermediate 1 (12.61 g, 19.8 mmol) in ethyl acetate (230 ml) and water (50 ml) is treated with a phase transfer catalyst (benzyltributylammonium chloride, 10 mol %), cooled to 3° C. and treated with bromofluoromethane (1.10 ml, 19.5 mmol, 0.98 equivalents), washing in with prechilled (0° C.) ethyl acetate (EtOAc) (20 ml). The suspension is stirred overnight, allowing to warm to 17° C. The aqueous layer is separated and the organic phase is sequentially washed with 1 M HCl (50 ml), 1%w/v $NaHCO_3$ solution (3×50 ml) and water (2×50 ml). The ethylacetate solution is distilled at atmospheric pressure until the distillate reaches a temperature of approximately 73° C. at which point toluene (150 ml) is added. Distillation is continued at atmospheric pressure until all remaining EtOAc has been removed (approximate distillate temperature 103° C.). The resultant suspension is cooled and aged at <10° C. and filtered off. The bed is washed with toluene (2×3 ml) and the product oven dried under vacuum at 60° C. to constant weight to yield the title compound (8.77 g, 82%) LCMS retention time 3.66 min, m/z 539 MH^+, NMR δ ($CDCl_3$) includes 7.60 (1H, m), 7.18-7.11 (2H, m), 6.52 (1H, dd, J 4.2 Hz), 6.46 (1H, s), 6.41 (1H, dd, J 10, 2Hz), 5.95 and 5.82 (2H dd, J51, 9 Hz), 5.48 and 5.35 (1H, 2m), 4.48 (1H, m), 3.48 (1H, m), 1.55 (3H, s), 1.16 (3H, s), 1.06 (3H, d, J 7 Hz).

Pharmacological Activity

In Vitro Pharmacological Activity

[0124] Pharmacological activity was assessed in a functional in vitro assay of glucocorticoid agonist activity which is generally predictive of anti-inflammatory or anti-allergic activity in vivo.

[0125] For tbe experiments in this section, compound of formula (I) was used as unsolvated Form 1 (Intermediate 2)

[0126] The functional assay was based on that described by K. P. Ray et al., Biochem J. (1997), 328, 707-715. A549 cells stably transfected with a reporter gene containing the NF-κB responsive elements from the ELAM gene promoter coupled to sPAP (secreted alkaline phosphatase) were treated with test compounds at appropriate doses for 1 hour at 37° C. The cells were then stimulated with tumour necrosis factor (TNF, 10 ng/ml) for 16 hours, at which time the amount of alkaline phosphatase produced is measured by a standard colourimetric assay. Dose response curves were constructed from which EC_{50} values were estimated.

[0127] In this test the compound of formula (I) showed an EC_{50}value of <1 nM.

[0128] The glucocorticoid receptor (GR) can function in at least two distinct mechanisms, by upregulating gene expression through the direct binding of GR to specific sequences in gene promotors, and by downregulating gene expression that is being driven by other transcription factors (such as NFκB or AP-1) through their direct interaction with GR.

[0129] In a variant of the above method, to monitor these functions, two reporter plasmids have been generated and introduced separately into A549 human lung epithelial cells by transfection. The first cell line contains the firefly luciferase reporter gene under the control of a synthetic promoter that specifically responds to activation of the transcription factor NFKκ when stimulated with TNFα. The second cell line contains the renilla luciferase reporter gene under the control of a synthetic promotor that comprises 3 copies of the consensus glucocorticoid response element, and which responds to direct stimulation by glucocorticoids. Simultaneous measurement of transactivation and transrepression was conducted by mixing the two cell lines in a 1:1 ratio in 96 well plate (40,000 cells per well) and growing overnight at 37° C. Test compounds were dissolved in DMSO, and added to the cells at a final DMSO concentration of 0.7%. After incubation for 1 h 0.5ng/ml TNFα (R&D Systems) was added and after a further 15 hours at 37° C., the levels of firefly and renilla luciferase were measured using the Packard Firelite kit following the manufacturers' directions. Dose response curves were constructed from which EC_{50} values were determined.

	Transactivation (GR) ED_{50} (nM)	Transrepression (NFκB) ED_{50} (nM)
Compound of Formula (I)	0.06	0.20
Metabolite (X)	>250	>1000
Fluticasone propionate	0.07	0.16

In Vivo Pharmacological Activity

[0130] Pharmacological activity in vivo was assessed in an ovalbumin sensitised Brown Norway rat eosinophilia model. This model is designed to mimic allergen induced lung eosinophilia, a major component of lung inflammation in asthma.

[0131] For the experiments in this section, compound of formula (I) was used as unsolvated Form 1.

[0132] Compound of formula (I) produced dose dependant inhibition of lung eosinophilia in this model after dosing as an intra-tracheal (IT) suspension in saline 30 min prior to ovalbumin challenge. Significant inhibition is achieved after a single dose of 30 μg of compound of formula (I) and the response was significantly (p=0.016) greater than that seen with an equivalent dose of fluticasone propionate in the same study (69% inhibition with compound of formula (I) vs 41% inhibition with fluticasone propionate).

[0133] In a rat model of thymus involution 3 daily IT doses of 100 μg of compound (I) induced significantly smaller reductions in thymus weight (p=0.004) than an equivalent dose of fluticasone propionate in the same study (67% reduction of thymus weight with compound (I) vs 78% reduction with fluticasone propionate).

[0134] Taken ther these results indicate a superior therapeutic index for compound (I) compared to fluticasone propionate.

In vitro Metabolism in Rat and Human Hepatocytes

[0135] Incubation of compound (I) with rat or human hepatocytes shows the compound to be metabolised in an identical manner to fluticasone propionate with the 17-βcarboxylic acid (X) being the only significant metabolite produced. Investigation of the rate of appearance of this metabolite on incubation of compound (I) with human hepatocytes (37° C., 10 μM drug concentration, hepatocytes from 3 subjects, 0.2 and 0.7 million cells/mL) shows compound (I) to be metabolised ca. 5-fold more rapidly than fluticasone propionate:

Subject number	Cell density (million cells/mL)	17-β acid metabolite production (pmol/h) Compound (I)	Fluticasone propionate
1	0.2	48.9	18.8
1	0.7	73.3	35.4
2	0.2	118	9.7
2	0.7	903	23.7
3	0.2	102	6.6
3	0.7	580	23.9

[0136] Median metabolite production **102-118** pmol/h for compound (I) and **18.8-23.0** pmol/h for fluticasone propionate.

Pharmacokinetics after Intravenous (IV) and Oral Dosing in Rats

[0137] Compound (I) was dosed orally (0.1 mg/kg) and IV (0.1 mg/kg) to male Wistar Han rats and pharmacokinetic parameters determined. Compound (I) showed negligible oral bioavailability (0.9%) and plasma clearance of 47.3 mL/min/kg, approaching liver blood flow (plasma clearance of fluticasone propionate=45.2 mL/min/kg).

Pharmacokinetics After Intra-tracheal Dry Powder Dosing in the Pig

[0138] Anaesthetised pigs (2) were dosed intra-tracheally with a homogenous mixture of compound (I) (1 mg) and fluticasone propionate (1 mg) as a dry powder blend in lactose (10% w/w). Serial blood samples were taken for up to 8h following dosing. Plasma levels of compound (I) and fluticasone propionate were determined following extraction and analysis using LC-MS/MS methodology, the lower limits of quantitation of the methods were 10 and 20 pg/mL for compound (I) and fluticasone propionate respectively. Using these methods compound (I) was quantifiable up to 2 hours after dosing and fluticasone propionate was quantifiable up to 8 hours after dosing. Maximum plasma concentrations were observed for both compounds within 15 min after dosing. Plasma half-life data obtained from IV dosing (0.1 mg/kg) was used to calculate AUC (0-inf) values for compound (I). This compensates for the plasma profile of Compound (I) only being defined up to 2 hours after an IT dose and removes any bias due to limited data between compound (I) and fluticasone propionate.

[0139] C_{max} and AUC (0-inf) values show markedly reduced systemic exposure to compound (I) compared to fluticasone propionate:

	Cmax (pg/mL) Pig 1	Pig 2	AUC (0-inf) (hr · pg/mL) Pig 1	Pig 2
Compound of Formula (I)	117	81	254	221
Fluticasone propionate	277	218	455	495

[0140] The pharmacokinetic parameters for both compound (I) and fluticasone propionate were the same in the anaesthetised pig following intravenous administration of a mixture of the two compounds at 0.1 mg/kg. The clearance of these two glucocorticoids is similar is this experimental pig model.

Examples

Example 1

Nasal Formulation Containing 6α, 9α-Difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid S-fluoromethyl ester

[0141] A formulation for intranasal delivery was prepared with ingredients as follows: 6α, 9α-Difluoro-17α-[(2-fura-

nylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta 1,4-diene-17β-carbothioic acid S-fluoromethyl ester 0.005% w/w

Tyloxapol	2% w/w
dextrose	5% w/w
BKC	0.015% w/w
EDTA	0.015% w/w
water	to 100%

[0142] in a total amount suitable for 120 actuations and the formulation was filled into a bottle (plastic or glass) fitted with a metering valve adapted to dispense 50 or 100 μl per actuation

[0143] The device was fitted into a nasal actuator (Valois, e.g. VP3, VP7 or VP7D)

[0144] The formulation was prepared as follows:

[0145] The surfactant Tyloxapol was first heated to 60-70° C. to lower its viscosity. Intermediate 2 was then added very slowly while stirring using a suitable propellor mixer, while the surfactant was still hot. Separately, approximately 80% remaining quantity of water was heated similarly to 60-70° C., and dextrose dissolved completely using a propellor mixer. This solution, while still hot, was added very slowly to the drug/Tyloxapol solution while stirring. This preparation was allowed to continue mixing for a minimum of 30 min, or until all drug was observed to dissolve completely. In the remaining water, BKC and EDTA were dissolved and then added slowly to the final formulation, which was further mixed until clear. If still necessary, the formulation was brought to its final weight with water alone. The pH was determined, and adjusted to pH 6.5 if necessary.

[0146] Similarly prepared were other formulations as follows:

Example 2

Nasal Formulation Containing 6α, 9α,-Difluoro-17α-[(2-furanylcarbonyl)oxyl-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid S-fluoromethyl ester

[0147] A formulation for intranasal delivery was prepared with ingredients as follows: 6α, 9α-Difluoro-17α-[(2-furanylcarbonyl)oxy]-11β-hydroxy-16α-methyl-3-oxo-androsta-1,4-diene-17β-carbothioic acid S-fluoromethyl ester 0.05% w/w

Triton X-100	5% w/w
Dextrose	4% w/w
BKC	0.015% w/w
EDTA	0.015% w/w
water	to 100%

[0148] in a total amount suitable for 120 actuations and the formulation was filled into a bottle fitted with a metering valve adapted to dispense 50 or 100 μl per actuation. The device was fitted into a nasal actuator (Valois).

Example 3

Nasal Formulation Containing Fluticasone Propionate

[0149] A formulation for intranasal delivery was prepared with ingredients as follows:

Fluticasone propionate	0.05% w/w
Triton X-100	5% w/w
Dextrose	4% w/w
BKC	0.015% w/w
EDTA	0.015% w/w
water	to 100%

[0150] in a total amount suitable for 120 actuations and the formulation was filled into a bottle fitted with a metering valve adapted to dispense 50 or 100 μl per actuation.

Example 4

Nasal Formulation Containing Fluticasone Propionate

[0151] A formulation for intranasal delivery was prepared with ingredients as follows:

Fluticasone propionate	0.05% w/w
Tyloxapol	5% w/w
dextrose	5% w/w
BKC	0.015% w/w
EDTA	0.015% w/w
water	to 100%

[0152] in a total amount suitable for 120 actuations and the formulation was filled into a bottle fitted with a metering valve adapted to dispense 50 or 100 μl per actuation The device was fitted into a nasal actuator (Valois).

Stability Testing

[0153] The chemical stability of Examples 1 and 2 was tested by placing samples at 5, 25 and 40° C. for a period 4 weeks and sampled as necessary. Analysis of the samples for drug content was done by HPLC.

Example	Condition	Drug amount (% label)	EDTA (% w/w)	BKC (% w/w)	pH
Example 1	Initial	97.1	0.0151	0.0146 (97.0)	6.70
	25° C./60% RH	94.2	0.0149	0.0144 (95.6)	6.20
	40° C./75% RH	92.3	0.0145	0.0150 (99.8)	5.82
Example 2	Initial	100.4	0.0157	0.0161 (107.3)	6.52
	25° C./60% RH	96.4	0.0151	0.0135 (90.3)	5.93
	40°c./75% RH	95.4	0.0149	0.0144 (96.1)	5.40

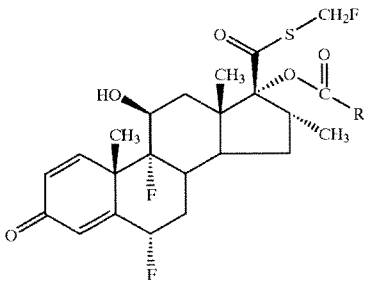
The data suggests that the examples were stable for a period of 1 month at accelerated conditions.

[0154] Throughout the specification and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer or step or group of integers but not to the exclusion of any other integer or step or group of integers or steps.

[0155] The patents and patent applications described in this application are herein incorporated by reference.

1. A pharmaceutical formulation comprising an aqueous carrier liquid having dissolved therein (a) an ester of fluticasone or a solvate thereof as medicament and (b) a solubilising agent for assisting the solubilisation of the medicament in the aqueous carrier liquid.

2. A pharmaceutical formulation according to claim 1 wherein the ester of fluticasone is a compound of formula (I)



(I)

wherein R represents ethyl or a 5 membered heterocyclic aromatic ring containing 1 to 3 heteroatoms selected from O, N and S, optionally substituted by one or more methyl or halogen atoms or a solvate thereof.

3. A pharmaceutical formulation according to claim 2 wherein R represents ethyl (i.e. the fluticasone ester is fluticasone propionate).

4. A pharmaceutical formulation according to claim 2 wherein R represents furan-2-yl.

5. A pharmaceutical formulation according to claim 1 wherein the solubilising agent is a surfactant.

6. A pharmaceutical formulation according to claim 5 wherein the surfactant is selected from the group consisting of α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly-(oxy-1,2-ethanediyl) polymer (also known as a octylphenoxypolyethoxyethanol) and a 4-(1,1,3,3-Tetramethylbutyl)phenol polymer with formaldehyde and oxirane.

7. A pharmaceutical formulation according to claim 4 wherein the solubilising agent is a surfactant selected from the group consisting of a α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly(oxy-1,2-ethanediyl) polymer (also known as a octylphenoxypolyethoxyethanol) and a 4-(1,1,3,3-Tetramethylbutyl)phenol polymer with formaldehyde and oxirane.

8. A pharmaceutical formulation according to claim 6 wherein the surfactant is a 4-(1,1,3,3-Tetramethylbutyl)phenol polymer with formaldehyde and oxirane.

9. A pharmaceutical formulation according to claim 1 which further has dissolved therein a hydroxy containing organic co-solvating agent or phosphatidyl choline.

10. A pharmaceutical formulation according to claim 7 which further has dissolved therein a hydroxy containing organic co-solvating agent or phosphatidyl choline.

11. A pharmaceutical formulation according to claim 9 wherein the the hydroxy containing organic co-solvating agent is dextrose.

12. A pharmaceutical formulation according to claim 10 wherein the the hydroxy containing organic co-solvating agent is dextrose.

13. A container containing a pharmaceutical formulation according to claim 1 fitted with a metering valve.

14. A device adapted for intranasal delivery of a pharmaceutical formulation comprising a container according to claim 13.

15. A method of treatment of inflammatory and/or allergic conditions of the nasal passages which comprises administering to the nose a pharmaceutical formulation according to claim 1.

* * * * *

APPENDIX C: RELATED PROCEEDINGS

No related proceedings are pending.